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INDIA  
1955

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VOLUME 24

SECTION—A

PART III

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PROCEEDINGS  
OF  
THE SYMPOSIUM  
ON  
RECENT TRENDS IN SOIL  
RESEARCH  
PART III

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ON  
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AT  
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ON  
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PART III



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PLAN FOR CONDUCTING SOIL EXPERIMENTAL FIELD WORK AT  
THE ALLAHABAD AGRICULTURAL INSTITUTE

By A. K. GHOSH, G. D. SINGH, and G. H. DUNGAN

*Agronomy Department, Allahabad Agricultural Institute*

(Received on 17th November, 1954)

The importance of growing a large amount of food has been repeatedly stressed in India. The production of *kharif* cereals—rice, *jowar*, *bajra*, and maize—was increased in 1952-53 over that of the previous year by 5.9 %. But this was accomplished by increasing the area sown to these crops by about 6 million acres or 5.5 %.<sup>1</sup> What we need is increased food production without the necessity of increasing the acreage. To do this a highly productive soil is necessary.

Long-time experiments to evaluate different soil management practices will provide information on how to make increased yields permanent. Though helpful at first, many people have experienced disappointment in the repeated use of chemical fertilizers containing nitrogen alone, and because of that they have questioned the advisability of using nitrogenous fertilizers at all. The correct answer to a question of this kind will come from carefully set-up field experiments. England has her Rothamsted and India should have a number of comparable soil experimental fields.

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1. Taken from India, *A reference Annual*, page 154. The Publication Division, Government of India, 1954.

The use of fertilizers is on the increase in India. This may properly be looked upon as a present day trend. To guide the village farmer in the wise use of fertilizers, many local soil experimental fields are needed. In an attempt to meet its share of this responsibility the Allahabad Agricultural Institute has undertaken field work with soils. In preparation for this the Institute has chosen a new location for its Agronomy Research Area. It was re-located in 1954 on a level tract of land which is south-east of the main buildings of the Institute adjoining the Rewah Road. A part of this space has been laid out into a soil experimental field. It consists of two series of plots, known as divisions 400 and 500, making it possible to grow two kinds of crops under the different soil treatments at the same time.

The plots have been treated as follows : (a) nothing applied as control, (b) ammonium sulphate, (c) superphosphate, and (d) potash. These have been used alone and in all possible combinations, making eight plots in each block. Each treatment is replicated four times. The individual plots are 9 feet wide and 36 feet long with an untreated border space on all sides. There is a three-foot space around the edge of the field or division and a space 6 or 9 feet between plots. The 6-foot spaces alternate with the 9-foot spaces which are to be used for irrigation channels during the *rabi* season.

The initial application of fertilizers was 200 pounds of ammonium sulphate, 250 pounds of 16 per cent. superphosphate, and 100 pounds of 50 per cent. potash per acre. The amount per acre is approximately the quantity of these fertilizing material contained in a moderately high yield of the common farm crops. Combinations of these materials were made, using the same amounts per acre. The purpose of the experiment is not to find out the amount of fertilizer to use, but to discover the kind or kinds of fertilizer needed. Another object, as indicated earlier, is to learn the effect of using the same kind of treatment repeatedly on the same land.

The soil on which these field experiments are located is a very fine sandy loam of alluvial origin. It is alkaline in reaction and for this reason treatment with limestone for correcting acidity was omitted. According to tests by A. U. Thor<sup>1</sup> on samples provided by F. H. Shuman<sup>2</sup> the pH was 7.8 and there were available more than 200 pounds of phosphorus and more than 300 pounds of potash per acre. These quantities of minerals are considered adequate for average crop yields. This is borne out by the results from a crop of maize planted across the variously treated plots, shown in Table 1.

---

1. A. U. Thor is in charge of the Soil Testing Laboratory, Illinois Agricultural Experiment Station, Urbana, Illinois.

2. H. F. Shuman was formerly Director of Training, Extension Short Courses, Allahabad Agricultural Institute.

The data show that the nitrogen had a pronounced effect up on the yield of ears per acre, upon the percentage of plants in silk on 26th August, upon the height of plants when fully tasseled, and upon the number of ears per plant. The use of phosphorus and potassium when applied separately and also when applied together did not alter the performance of the plants significantly from the control.

TABLE I

Data from a crop of Jaunpur yellow flint maize grown on plots receiving different fertilizing materials at the Allahabad Agricultural Institute, 1954.

Materials applied to the soil	Yield of ears (grain in soft dough stage) per acre	Yield of green stover per acre	Plants in silk on 26th August, 1954	Height of plants when fully tas- seled	Ears per plant
	Maunds	Maunds	Per cent.	Inches	Number
N P K	40.0	67.1	55.3	66.3	0.87
N K	38.2	70.3	53.2	61.9	0.78
N P	36.9	59.7	48.6	65.4	0.98
N	36.7	68.5	55.9	64.0	0.80
P	28.7	54.9	31.0	56.7	0.64
None	24.4	46.7	28.6	56.4	0.65
P K	18.9	47.4	27.0	52.1	0.51
K	12.0	33.8	13.7	48.4	0.45
Average	29.5	56.1	39.2	58.9	0.71
L. S. D. @5%	26.2	N. S.	26.1	15.7	0.31

The variability of the crop on plots treated alike is reflected in the large "least significant difference" value shown at the bottom of the columns of data. This field had been included as a part of the general farm for many years and the soil fertility replenishment practice had not been uniform. The plan for the future as indicated previously, is to re-treat these plots with the same fertilizing materials prior to the sowing of each crop and thus build up a surplus of some plant foods and create a deficit of others.

## SUMMARY

1. The Allahabad Agricultural Institute has chosen a new location for its Agronomy Research Field. It is on a level tract of land near the campus and adjoining the Rewah Road.

2. A long time experiment involving soil treatment with different fertilizers was put into operation in 1954.

3. The objectives of the project are (a) to discover the kinds of fertilizer needed for the production of good yields of farm crops, and (b) to find out what the continued treatment of the soil with the materials being used will have on crop response.

4. Preliminary trials with maize showed that this crop made its greatest growth on soils treated with a nitrogenous fertilizer.

5. In general, however, maize yields were highest on plots receiving the full treatment of nitrogen, phosphorus and potassium.

# INFLUENCE OF DISODIUM HYDROGEN PHOSPHATE AND CALCIUM CARBONATE ON ATMOSPHERIC NITROGEN FIXATION UNDER STERILE CONDITION

By. N R DHAR AND S. K. GHOSH

*Sheila Dhar Institute of Soil Science, Allahabad University, Allahabad*

(Received on 15th November, 1954)

The use of phosphates in reinforcing manures is well-known and Bear<sup>1</sup> has remarked that "within limits phosphate fertilisers together with potash salts and lime can be substituted for nitrogen fertilisers. Their use stimulates the nitrogen fixing bacteria, both symbiotic and non-symbiotic, to greater activity."

Dhar<sup>2</sup> has reported that by the use of lime or calcium carbonate to an acid soil, not only the acidity decreases but also the carbonaceous substances undergo more rapid oxidation leading to the fixation of atmospheric nitrogen in soil so that the C/N ratio decreases and its fertility increases. Recently, Dhar<sup>3</sup> and co-workers have further shown that the amount of nitrogen fixed per gram of carbon oxidised when energy rich materials are mixed with soil or sand is greatly intensified if the soil or sand is rich in calcium phosphate. When wheat straw in a finely divided condition is allowed to undergo oxidation in a soil containing 0.043% nitrogen, 0.992% CaO and 0.078%  $P_2O_5$ , the amount of nitrogen fixed per gram of carbon oxidised is 25 m. gm. in light and about 12 m. gm. in the dark. When the soil contains 0.2% N, 3.4% CaO and 0.42%  $P_2O_5$ , the amount of nitrogen fixed is about 6 times greater than that obtained in the previous soil. These observations clearly indicate the importance of phosphates, and calcium carbonate in maintaining the nitrogen status of soils.

In this paper we have studied the influence of both disodium hydrogen phosphate and calcium carbonate on nitrogen fixation effected by the slow oxidation of energy rich materials such as glucose, cane sugar and starch on the surfaces of some pure inorganic materials like ZnO,  $MnO_3$ ,  $Fe_2O_3$  and  $TiO_3$ . The experiments have been conducted in completely sterile condition to avoid any biological process taking part in this type of nitrogen fixation.

## EXPERIMENTAL

The energy rich materials and the solid surfaces used were of Merck's reagent. The reaction mixtures were taken in long Jena glass test-tubes. The total volume of the solution in the test-tubes, in all cases, was kept to 10 cc. which was made up by adding conductivity water where necessary. Experiments were conducted in completely sterile condition both in presence and absence of either disodium hydrogen phosphate or calcium carbonate. The concentration of disodium hydrogen phosphate and calcium carbonate added in all cases was 0.25 gm.  $P_2O_5$  as  $Na_2HPO_4$  and 0.25 gm.  $CaCO_3$  respectively per 100 grams of the solid surfaces used. Sterilisation, in all cases, was carried out in an autoclave at 10 lbs pressure for 3 hours. One set of test-tubes containing the reaction mixtures was

exposed to light from a 60 Watt electric bulb working on 220Volts and kept at a distance of 2½ feet from the bulb whilst another set kept at the same place was covered with thick black cloth. The exposure was continued day and night and the test-tubes occasionally shaken to facilitate aeration. The average mean temperature was recorded during the exposure.

At a definite interval of time the solution in the test-tubes was carefully evaporated to dryness on a waterbath with a drop of dilute sulphuric acid and then the whole test tube along with the dried mass was broken into a Kjeldahl flask and finally charred with concentrated sulphuric acid for the estimation of total carbon and total nitrogen. The total carbon and the total nitrogen was estimated according to the method of Robinson, McLean and Williams.<sup>4</sup> The experimental results are as follows :—

TABLE No. 1

Average Temperature = 27°C

<i>Sterile</i>		<i>Light</i>			
1 gm. ZnO + Glucose Per 100 gms. of Zinc oxide					
Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm.	Total nitrogen present in gm.	Gain in total nitrogen in gm.	Efficiency
0	1.5210	0	0	0	...
210	1.3165	0.2045	0.0078	0.0078	38.14
1 gm ZnO + Glucose + Phosphate					
0	1.5210	0	0	0	...
210	1.2349	0.2861	0.0140	0.0140	48.93
1 gm ZnO + Calcium carbonate					
0	1.5210	0	0	0	...
210	1.1655	0.3555	0.0150	0.0150	42.19

TABLE No. 2

<i>Sterile</i>		<i>Dark</i>			
		1 gm ZnO + Glucose			
0	1.5210	0	0	0	
210	1.4213	0.0997	0.0024	0.0024	24.07
		1 gm ZnO + Glucose + Phosphate			
0	1.5210	0	0	0	
210	1.3456	0.1754	0.0055	0.0055	31.36
		1 gm ZnO + Glucose + Calcium carbonate			
0	1.5210	0	0	0	
210	1.2757	0.2453	0.0066	0.0066	26.90



TABLE No. 3

Average Temperature = 27°C

*Sterile**Light*1 gm  $\text{Fe}_2\text{O}_3$  + Glucose

Per 100 gms of Ferric oxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.5210	0	0	0	...
210	1.2404	0.2806	0.0099	0.0099	35.28
1 gm $\text{Fe}_2\text{O}_3$ + Glucose + Phosphate					
0	1.5210	0	0	0	...
210	1.1827	0.3383	0.0156	0.0156	46.11
1 gm $\text{Fe}_2\text{O}_3$ + Glucose + Calcium carbonate					
0	1.5210	0	0	0	...
210	1.1192	0.4018	0.0165	0.0165	41.06

TABLE No. 4

*Sterile**Dark*1 gm  $\text{Fe}_2\text{O}_3$  + Glucose

0	1.5210	0	0	0	...
210	1.3270	0.1940	0.0042	0.0042	21.65

1 gm  $\text{Fe}_2\text{O}_3$  + Glucose + Phosphate

0	1.5210	0	0	0	...
210	1.2577	0.2633	0.0080	0.0080	30.39

1 gm  $\text{Fe}_2\text{O}_3$  + Glucose + Calcium carbonate

0	1.5210	0	0	0	...
210	1.2058	0.3152	0.0083	0.0083	26.33

TABLE No. 5

Average Temperature = 27°C

*Sterile**Light*1 gm MnO<sub>2</sub> + Glucose

Per 100 gm of Manganese dioxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.5210	0	0	0	...
210	0.3173	1.2037	0.0170	0.0170	14.12
1 gm MnO <sub>2</sub> + Glucose + Phosphate					
0	1.5210	0	0	0	...
210	0.2581	1.2629	0.0221	0.0221	17.49
1 gm MnO <sub>2</sub> + Glucose + Calcium carbonate					
0	1.5210	0	0	0	...
210	0.1962	1.3248	0.0211	0.0211	15.93

TABLE No. 6

*Sterile**Dark*1 gm MnO<sub>2</sub> + Glucose

0	1.5210	0	0	0	...
210	0.4327	1.0883	0.0104	0.0104	9.56
1 gm MnO <sub>2</sub> + Glucose + Phosphate					
0	1.5210	0	0	0	...
210	0.3461	1.1749	0.0150	0.0150	12.77
1 gm MnO <sub>2</sub> + Glucose + Calcium carbonate					
0	1.5210	0	0	0	...
210	0.2819	1.2391	0.0145	0.0145	11.70

TABLE No. 7

Average Temperature = 27°C

*Sterile**Light*1 gm  $\text{TiO}_2$  + Glucose

Per 100 gms of Titanium dioxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.5210	0	0	0	...
210	1.3769	0.1441	0.0055	0.0055	38.12
1 gm $\text{TiO}_2$ + Glucose + Phosphate					
0	1.5210	0	0	0	...
210	1.2715	0.2495	0.0121	0.0121	48.49
1 gm $\text{TiO}_2$ + Glucose + Calcium carbonate					
0	1.5210	0	0	0	...
210	1.2150	0.3060	0.0130	0.0130	42.48

TABLE No. 8

*Sterile**Dark*1 gm  $\text{TiO}_2$  + Glucose

0	1.5210	0	0	0	...
210	1.4719	0.0491	0.0011	0.0011	22.40

1 gm  $\text{TiO}_2$  + Glucose + Phosphate

0	1.5210	0	0	0	...
210	1.4010	0.1200	0.0035	0.0035	29.17

1 gm  $\text{TiO}_2$  + Glucose + Calcium carbonate

0	1.5210	0	0	0	...
210	1.3365	0.1845	0.0045	0.0045	24.39

TABLE No. 9

Average Temperature = 27°C

*Sterile**Ligh*

1 gm ZnO + Cane Sugar

Per 100 gm of Zinc oxide

Period of expoure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.5267	0	0	0	...
230	1.3219	0.2048	0.0072	0.0072	35.16
1 gm ZnO + Cane Sugar + Phosphate					
0	1.5267	0	0	0	...
230	1.2451	0.2816	0.0134	0.0134	47.59
1 gm ZnO + Cane Sugar + Calcium carbonate					
0	1.5267	0	0	0	...
230	1.1759	0.3508	0.0143	0.0143	40.76

TABLE No. 10

*Sterile**Dark*

1 gm ZnO + Cane Sugar

0	1.5267	0	0	0	...
230	1.4270	0.0997	0.0021	0.0021	21.06
1 gm ZnO + Cane Sugar + Phosphate					
0	1.5267	0	0	0	...
230	1.3508	0.1699	0.0048	0.0048	28.25
1 gm ZnO + Cane Sugar + Calcium carbonate					
0	1.5267	0	0	0	...
230	1.2892	0.2375	0.0057	0.0057	24.00

TABLE No. 11

Average Temperature=27°C

*Sterile**Light*

1 gm of Cane Sugar  
Per 100 gm of Ferric oxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.5267	0	0	0	...
230	1.2515	0.2752	0.0090	0.0090	32.70
1 gm Fe <sub>2</sub> O <sub>3</sub> +Cane Sugar+Phosphate					
0	1.5267	0	0	0	...
230	1.1931	0.3336	0.0149	0.0149	44.66
1 gm Fe <sub>2</sub> O <sub>3</sub> +Cane Sugar+Calcium carbonate					
0	1.5267	0	0	0	...
230	1.1295	0.3972	0.0158	0.0158	39.78

TABLE No. 12

*Sterile**Dark*

0	1.5267	0	0	0	...
230	1.3281	0.1986	0.0036	0.0036	18.13
1 gm Fe <sub>2</sub> O <sub>3</sub> +Cane Sugar+Phosphate					
0	1.5267	0	0	0	...
230	1.2681	0.2586	0.0074	0.0074	28.62
1gm Fe <sub>2</sub> O <sub>3</sub> +Cane Sugar+Calcium carbonate					
0	1.5267	0	0	0	...
230	1.2194	0.3073	0.0075	0.0075	24.43

TABLE No. 13

Average Temperature = 27°C

*Sterile**Light*1 gm MnO<sub>2</sub> + Cane Sugar  
Per 100 gms. of manganese dioxide

Period of exposure days	Total carbon pre- sent in gm.	Total carbon oxidis- ed in gm.	Total nitrogen present in gm.	Gain in total nitrogen in gm	Efficiency
0	1.5267	0	0	0	...
230	0.3276	1.1991	0.0162	0.0162	13.51
1 gm MnO <sub>3</sub> + Cane Sugar + Phosphate					
0	1.5267	0	0	0	...
230	0.2685	1.2582	0.0214	0.0214	17.08
1 gm MnO <sub>3</sub> + Cane Sugar + Caliaum carbonate					
0	1.5267	0	0	0	...
230	0.2065	1.3202	0.0203	0.0203	15.38

TABLE No. 14

*Sterile**Dark*1 gm MnO<sub>2</sub> + Cane Sugar

0	1.5267	0	0	0	...
230	0.4435	1.0832	0.0095	0.0095	8.77
1 gm MnO <sub>2</sub> + Cane Sugar + Phosphate					
0	1.5267	0	0	0	...
230	0.3570	1.1697	0.0142	0.0142	12.14
1 gm. MnO <sub>2</sub> + Cane Sugar + Calcium carbonate					
0	1.5267	0	0	0	...
230	0.2924	1.2343	0.0138	0.0138	11.18

TABLE No. 15

Average Temperature = 27°C

*Sterile**Light*1 gm  $\text{TiO}_2$  + Cane Sugar

Per 100 gms of Titanium dioxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm.	Gain in total nitrogen in gm	Efficiency
----------------------------	----------------------------	-----------------------------	-------------------------------	------------------------------	------------

0	1.5267	0	0	0	...
230	1.3871	0.1396	0.0049	0.0049	35.10

1 gm  $\text{TiO}_2$  + Cane Sugar - Phosphate

0	1.5267	0	0	0	...
230	1.2820	0.2447	0.0114	0.0114	46.59

1 gm  $\text{TiO}_2$  + Cane Sugar + Calcium Carbonate

0	1.5267	0	0	0	...
230	1.2258	0.3009	0.0124	0.0124	41.21

TABLE No. 16

*Sterile**Dark*1 gm  $\text{TiO}_2$  + Cane Sugar

0	1.5267	0	0	0	...
230	1.4830	0.0437	0.0009	0.0009	20.59

1 gm  $\text{TiO}_2$  + Cane Sugar + Phosphate

0	1.5267	0	0	0	...
230	1.4142	0.1125	0.0028	0.0028	24.89

1 gm  $\text{TiO}_2$  + Cane Sugar + Calcium carbonate

0	1.5267	0	0	0	...
230	1.3491	0.1776	0.0038	0.0038	21.39

TABLE No. 17

Average Temperature = 27°C

*Sterile**Light*

1 gm ZnO + Starch

Per 100 gms of Zinc oxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in nitrogen in gm	Efficiency
0	1.3366	3	0	0	...
240	1.1520	0.1846	0.0056	0.0056	30.34
1 gm ZnO + Starch + Phosphate					
0	1.3366	0	0	0	...
240	1.0750	3.2616	0.0117	0.0117	44.72
1 gm ZnO + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	1.0058	0.3308	0.0125	0.0125	37.79

TABLE No. 18

*Sterile**Dark*

1 gm ZnO + Starch

0	1.3366	0	0	0	...
240	1.2596	0.0770	0.0014	0.0014	18.18

1 gm ZnO + Starch + Phosphate

0	1.3366	0	0	0	...
420	1.1909	0.1457	0.0032	0.0032	21.96

1 gm ZnO + Starch + Calcium carbonate

0	1.3366	0	0	0	...
240	1.1309	0.2057	0.0040	0.0040	19.25



TABLE No. 19

Average Temperature = 27°C

*Sterile**Light*1 gm  $\text{Fe}_2\text{O}_3$  + Starch

Per 100 gm of Ferric oxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.3366	0	0	0	...
240	1.0815	0.2551	0.0074	0.0074	29.01
1 gm $\text{Fe}_2\text{O}_3$ + Starch + Phosphate					
0	1.3366	0	0	0	...
240	1.0230	0.3136	0.0135	0.0135	43.05
1 gm $\text{Fe}_2\text{O}_3$ + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	0.9593	0.3773	0.0140	0.0140	37.16

TABLE No. 20

*Sterile**Dark*1 gm  $\text{Fe}_2\text{O}_3$  + Starch

0	1.3366	0	0	0	...
240	1.1678	0.1688	0.0025	0.0025	14.81

1 gm  $\text{Fe}_2\text{O}_3$  + Starch + Phosphate

0	1.3366	0	0	0	...
240	1.1095	0.2271	0.0058	0.0058	25.54

1 gm  $\text{Fe}_2\text{O}_3$  + Starch + Calcium carbonate

0	1.3366	0	0	0	...
240	1.0595	0.2771	0.0056	0.0056	20.21

TABLE No. 21

Average Temperature = 27°C

*Sterile**Light*1 gm MnO<sub>2</sub> + Starch

Per 100 gm of manganese dioxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.3366	0	0	0	...
240	0.1575	1.1791	0.0145	0.0145	12.29
1 gm MnO <sub>2</sub> + Starch + Phosphate					
0	1.3366	0	0	0	...
240	0.0984	1.2382	0.0198	0.0198	15.99
1 gm MnO <sub>2</sub> + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	0.0365	1.3001	0.0186	0.0186	14.31

TABLE No. 22

*Sterile**Dark*1 gm MnO<sub>2</sub> + Starch

0	1.3366	0	0	0	...
240	0.2749	1.0617	0.0079	0.0079	7.44
1 gm MnO <sub>2</sub> + Starch + Phosphate					
0	1.3366	0	0	0	...
240	0.1882	1.1484	0.0127	0.0127	11.06
1 gm MnO <sub>2</sub> + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	0.1235	1.2131	0.0120	0.0120	10.72

TABLE No. 23

*Sterile**Light*

Average Temperature = 27°C

1 gm  $\text{TiO}_2$  + Starch

Per 100 gms of Titanium dioxide

Period of exposure in days	Total carbon present in gm	Total carbon oxidised in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
0	1.3366	0	0	0	...
240	1.2172	0.1194	0.0034	0.0034	28.48
1 gm $\text{TiO}_2$ + Starch + Phosphate					
0	1.3366	0	0	0	...
240	1.1120	0.2246	0.0098	0.0098	43.63
1 gm $\text{TiO}_2$ + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	1.0558	0.2808	0.0109	0.0109	38.82

TABLE No. 24

*Sterile**Dark*1 gm  $\text{TiO}_2$  + Starch

0	1.3366	0	0	0	...
240	1.3142	0.0224	0.0004	0.0004	17.86
1 gm $\text{TiO}_2$ + Starch + Phosphate					
0	1.3366	0	0	0	...
240	1.2451	0.0915	0.0019	0.0019	20.77
1 gm $\text{TiO}_2$ + Starch + Calcium carbonate					
0	1.3366	0	0	0	...
240	1.1820	0.1546	0.0029	0.0029	18.76

The foregoing experimental results clearly show that when energy rich materials like glucose, cane sugar and starch are allowed to undergo slow oxidation in air in contact with the solid surfaces of zinc oxide, ferric oxide, manganese dioxide and titania in presence or absence of either disodium hydrogen phosphate or calcium carbonate, then, along with the decrease of the carbon content in the system, there is a concomitant increase in the total nitrogen. As the experiments have been carried out in sterile conditions, the nitrogen fixation has taken place without any bacterial action. There is always greater fixation of nitrogen in light than in the dark showing marked effect of light absorption in nitrogen fixation of this type.

Again it will be seen from these results that in all cases the presence of either disodium hydrogen phosphate or calcium carbonate increases the oxidation of the energy materials and the efficiency of nitrogen fixation per gram of carbon oxidised. The oxidation however, is greater in presence of calcium carbonate than with disodium hydrogen phosphate. In the following tables are given the percentage increase in the efficiency of nitrogen fixation both with disodium hydrogen phosphate and calcium carbonate in light as well as in the dark.

TABLE No. 25

*Sterile*

Average Temperature = 27°C

			Percentage increase in efficiency after 210 days	
			With Phosphate	With Calcium carbonate
1.	ZnO + Glucose	Light	28.29	10.62
		Dark	30.29	11.76
2.	Fe <sub>2</sub> O <sub>3</sub> + Glucose	Light	29.42	16.38
		Dark	40.37	21.62
3.	MnO <sub>2</sub> + Glucose	Light	23.84	12.82
		Dark	33.58	22.39
4.	TiO <sub>2</sub> + Glucose	Light	27.23	11.44
		Dark	30.22	8.88

TABLE No. 26

*Sterile*

Average Temperature = 27°C

			Percentage increase in efficiency after 230 days	
			With Phosphate	With Calcium carbonate
1.	ZnO + Cane sugar	Light	35.35	15.93
		Dark	34.14	13.96
2.	Fe <sub>2</sub> O <sub>3</sub> + Cane sugar	Light	36.58	21.65
		Dark	57.86	34.75
3.	MnO <sub>2</sub> + Cane sugar	Light	26.42	13.84
		Dark	38.43	27.48
4.	TiO <sub>2</sub> + Cane sugar	Light	32.73	17.41
		Dark	20.88	3.88

TABLE No. 27

*Strile*

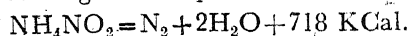
Average Temperature = 27°C

			Percentage increase in efficiency after 240 days	
			with Phosphate	with Calcium carbonate
1.	ZnO + Starch	Light	47.39	24.55
		Dark	20.79	5.88
2.	Fe <sub>2</sub> O <sub>3</sub> + Starch	Light	48.39	28.09
		Dark	72.45	36.46
3.	MnO <sub>2</sub> + Starch	Light	30.10	16.44
		Dark	48.66	44.08
4.	TiO <sub>2</sub> + Starch	Light	53.19	36.30
		Dark	16.29	5.06

The foregoing results show that in presence of disodium hydrogen phosphate and calcium carbonate there is an increase in the efficiency of nitrogen fixation. The percentage increase in efficiency in all cases is more pronounced with disodium hydrogen phosphate than with calcium carbonate. It cannot, however, be definitely concluded from these results that the effect of disodium hydrogen phosphate or calcium carbonate in increasing the efficiency of nitrogen fixation is more marked in light or in the dark.

## DISCUSSION

It is interesting to record that as soon as some protein or amino acids or ammonium salts are produced in the process of the fixation of atmospheric nitrogen, these nitrogenous compounds also undergo oxidation causing the formation and decomposition of the unstable substance ammonium nitrite which readily loses nitrogen according of the equation:



Hence, the nitrogenous compounds formed cannot remain unchanged for a long time. In other words, as the process of nitrogen fixation continues and the oxidation of carbohydrates or other energy materials proceeds far, the efficiency of the process falls off. Thus, when the loss during nitrification is prominent, the apparent fixation becomes less efficient. It is clear, therefore, that if the carbohydrate oxidation has proceeded far, *i.e.*, the amount of oxidation of the carbon has been large, the apparent efficiency of the process is lower than when oxidation of the energy material is comparatively small. This is evident from our results obtained with manganese dioxide as the surface which is certainly more effective in increasing the oxidation of carbohydrates than with the other oxide surfaces during the same period of exposure. Hence, the efficiency of nitrogen fixation with manganese dioxide surface is much smaller than with other oxide surfaces. The above explanation can throw light why we have obtained, in some cases, less percentage increase in efficiency with disodium hydrogen phosphate and calcium carbonate in light than in the dark. Here, the loss of nitrogen due to the formation and decomposition of ammonium nitrite in light becomes prominent along with fixation of nitrogen.

The part played by phosphates and calcium carbonate will be clear if we consider their dissociation constants :

Carbonic acid :  $\text{H}_2\text{CO}_3$  : dissociation constant  $= 3 \times 10^{-7}$

$$\frac{\text{H}^+ \times \text{HCO}_3'}{\text{H}_2\text{CO}_3} = 3 \times 10^{-7} \text{ (first dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{CO}_3''}{\text{HCO}_3'} = 6 \times 10^{-11} \text{ (second dissociation constant)}$$

Phosphoric acid :  $\text{H}_3\text{PO}_4$  : dissociation constant  $= 9 \times 10^{-8}$

$$\frac{\text{H}^+ \times \text{H}_2\text{PO}_4'}{\text{H}_3\text{PO}_4} = 1.1 \times 10^{-2} \text{ (first dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{HPO}_4''}{\text{H}_2\text{PO}_4'} = 2 \times 10^{-7} \text{ (second dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{PO}_4'''}{\text{HPO}_4''} = 3.6 \times 10^{-13} \text{ (third dissociation constant)}$$

Because the second and third dissociation constants of phosphoric acid are small, whenever hydrogen ions are available in the system during the course of oxidation of the organic compounds in contact with the inorganic materials used in our experiments, along with phosphate ions,  $\text{HPO}_4''$  and  $\text{H}_2\text{PO}_4'$  ions are formed and hence the hydrogen ion concentration in the system decreases. Similarly as the first and second dissociation constants of carbonic acid are also small, carbonate and bicarbonate ions present in the system readily combine with hydrogen ions and form carbonic acid. Thus phosphates and carbonates when present along with the decomposing organic matter can act as excellent buffers and do not allow the increase in the hydrogen ion concentration in the system and hence the

decomposition of the unstable substance ammonium nitrite, which is always produced in the process of the fixation of atmospheric nitrogen, is decreased. It is well-known that the decomposition of ammonium nitrite is catalysed positively by acids and retarded in presence of alkali. Hence, it can be inferred that by the use of phosphates or calcium carbonate not only the acidity of the system decreases but also the carbonaceous matter undergoes more rapid oxidation. It is well-known that alkali favours oxidation of carbohydrates and reduces the decomposition of ammonium nitrite. Hence in presence of carbonate and phosphates the loss of nitrogen gas is retarded and hence a higher efficiency of nitrogen fixation in presence of disodium hydrogen phosphate or calcium carbonate both in light as well as in the dark is observed. Dhar<sup>5</sup> has also remarked as follows : "In presence of large amounts of phosphate in the system more or less stable phosphoproteins are formed by the combination of proteins and phosphorus compounds. These compounds seem to resist nitrification and ammonification and loss of nitrogen better than proteins alone. This appears to be an important reason why the efficiency of nitrogen fixation in soils, both under sterile and unsterile conditions, appears larger in presence of increasing quantities of phosphates."

From the foregoing results showing marked increase of nitrogen fixation in the oxidation of organic substances aided by disodium hydrogen phosphate or calcium carbonate, it is convincing that their use in soils will lead to marked nitrogen fixation not only in the acid soils of the world but also to soils rich in organic matter as found in many places of Europe and America. It is well-known that phosphates intensify the growth of micro-organisms and, hence, nitrogen fixation can be increased by the addition of phosphates. But our experimental results recorded in the foregoing pages, with different surfaces containing phosphate and calcium carbonate under completely sterile conditions also show that nitrogen fixed per gram of carbon oxidised is much greater in presence of phosphate and calcium carbonate than in their absence. Hence, the use of phosphates or calcium carbonate in soils in presence of decomposing organic matter is of double benefit, firstly, as agent, for increasing the nitrogen status of a soil, and secondly, by their vital importance as plant nutrients. These results therefore, are of great significance in improving soil fertility.

It is clear, therefore, that a mixture of organic substances like farmyard manure, straw, plant leaves, grass, etc., fortified by addition of calcium phosphate should prove to be of immense value in building up soil fertility permanently by decreasing acidity, fixing atmospheric nitrogen and supplying available phosphate, potash and trace elements. Hence it is always desirable to increase the calcium phosphate reserve of soils in permanent agriculture.

#### SUMMARY

1. The energy materials like glucose, cane sugar and starch mixed with oxides like  $\text{ZnO}$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{MnO}_2$ ,  $\text{TiO}_2$  have been found to fix the nitrogen of air even under completely sterile conditions. The amount of nitrogen fixed per gram of carbon oxidised in sets exposed to artificial light from a 60 watt electric bulb is of the order of about 35 milligrams whilst it is approximately 20 milligrams in similar sets covered with thick black cloth. It appears, therefore, that apart from other considerations light plays an important part in the fixation of atmospheric nitrogen.

2. Presence of either disodium hydrogen phosphate or calcium carbonate increases carbon oxidation and the efficiency of nitrogen fixation both in light as well as in the dark. In presence of disodium hydrogen phosphate the efficiency is of the order of 45 milligrams in light and 30 milligrams in dark whilst in presence of calcium carbonate the efficiency is about 40 milligram in light and 26 milligrams in dark. This may be due to the fact that the process of nitrogen fixation is opposed by the phenomenon of nitrogen loss caused by the oxidation of proteins and ammonium salts to ammonium nitrite which readily loses nitrogen, but, in presence of phosphates and calcium carbonate, which act as buffers, the hydrogen ion concentration of the system is not allowed to increase and hence the decomposition of ammonium nitrite is checked.

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# NITROGEN LOSS IN SOILS ON THE APPLICATION OF NITROGENOUS COMPOUNDS AND ITS RETARDATION BY THE ADDITION OF POTASSIUM CHLORIDE

By N. R. DHAR and S. K. GHOSH

*Sheila Dhar Institute of Soil Science, Allahabad University, Allahabad*

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In previous publications<sup>1,2</sup> from this laboratory it has been shown that marked losses of nitrogen take place when ammonium salts and other nitrogenous compounds undergo nitrification in the soil or in solution. This loss appears to be unavoidable in the soil under normal conditions of cultivation. Field trials with different manures at Rothamsted<sup>3</sup> and elsewhere reveal that approximately 70% of the added nitrogenous manure may be lost without benefit to the soil or the crop.

We have carried on further experiments on this type of nitrogen loss under aerobic conditions with ammonium sulphate and urea when they undergo nitrification in soil and its retardation by adding potassium chloride.

## EXPERIMENTAL

100 grams of well-dried and powdered good quality soil (screened through a 100 mesh sieve) were accurately weighed and taken in shallow enamelled dishes. Amounts of urea or ammonium sulphate (A. R. quality), each containing exactly 1.00 gm nitrogen were mixed with the soil with and without different doses of potassium chloride. The concentrations of potassium in potassium chloride added were 2 gm, 5 gm and 10 gm per 100 grams of soil respectively. The contents of the dishes were thoroughly mixed and samples were taken out for analysis for their initial carbon, nitrogen, ammoniacal and nitrate nitrogen contents. Two sets of each mixture were made, one was kept exposed to light of an electric bulb of 60 watts working on 220 volts at a distance of 3 feet from the bulb whilst the other was kept covered with thick black cloth by the side of the exposed one. The mixtures were stirred carefully by means of a glass rod on alternate days and their moisture content was maintained at nearly 25% by adding distilled water. The light exposure was continued day and night and samples were taken out at regular intervals for analysis of their total carbon, total nitrogen, ammoniacal and nitrate nitrogen contents. The total carbon was estimated according to the method of Robinson, McLean and Williams<sup>4</sup> and the total nitrogen by Kjeldahl's method<sup>5</sup> the average mean temperature was recorded during the exposure. The following experimental results have been obtained :—

TABLE I  
Percentage Composition of the oven-dried soil

pH of the soil	...	8.00
Total Carbon	...	1.86
Total Nitrogen	...	0.26785
NH <sub>3</sub> -N	...	0.00608
NO <sub>3</sub> -N	...	0.02030
CaO	...	4.07
K <sub>2</sub> O	...	1.00
P <sub>2</sub> O <sub>5</sub>	...	0.42

TABLE 2  
Average Temperature = 25°C  
*Light* (Unsterile)

Treatment		Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per 100 grams of the mixture		
			After 15 days	After 30 days	After 60 days
1.	100 gm soil +	NH <sub>3</sub> -N = 0.96080	0.28570	0.17780	0.09850
	1.0 gm N as	NO <sub>3</sub> -N = 0.01940	0.04550	0.05880	0.08890
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Total-N = 1.21077	0.53800	0.43850	0.40200
		Total-C = 1.77630	1.51200	1.47000	1.39800
2.	100 gm soil +	NH <sub>3</sub> -N = 0.92700	0.36360	...	...
	1.0 gm N as	NO <sub>3</sub> -N = 0.01870	0.04940	...	...
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 2	Total-N = 1.16820	0.65410	...	...
	gm				
3.	K as KCl	Total-C = 1.71380	1.49410	...	...
	100 gm soil +	NH <sub>3</sub> -N = 0.88060	0.51080	0.38100	0.22230
	1.0 gm N as	NO <sub>3</sub> -N = 0.01780	0.05410	0.07140	0.10250
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 5	Total-N = 1.10960	0.77450	0.60670	0.55840
4.	gm				
	K as KCl	Total-C = 1.62790	1.47600	1.44000	1.36820
	100 gm soil +	NH <sub>3</sub> -N = 0.81270	0.57140	0.44450	0.33340
	1.0 gm N as	NO <sub>3</sub> -N = 0.01640	0.06450	0.08333	0.12120
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 10	Total-N = 1.02410	0.91380	0.72970	0.68910
	gm				
	K as KCl	Total-C = 1.50240	1.44650	1.41050	1.34410

TABLE 3  
Average Temperature 25°C  
*Dark* (Unsterile)

1.	100 gm soil +	NH <sub>3</sub> -N = 0.96080	0.30770	0.20510	0.13333
	1.0 gm N as	NO <sub>3</sub> -N = 0.01940	0.04880	0.06250	0.09520
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +	Total-N = 1.21077	0.59990	0.49670	0.44530
		Total-C = 1.77630	1.54230	1.53000	1.49400
2.	100 gm soil +	NH <sub>3</sub> -N = 0.92700	0.38690	...	...
	1.0 gm N as	NO <sub>3</sub> -N = 0.01870	0.05260	...	...
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 2	Total-N = 1.16820	0.72190	...	...
	gm				
3.	K as KCl	Total-C = 1.71380	1.52400	...	...
	100 gm soil +	NH <sub>3</sub> -N = 0.88060	0.54050	0.41600	0.26670
	1.0 gm N as	NO <sub>3</sub> -N = 0.01780	0.05880	0.07690	0.11112
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 5	Total-N = 1.10960	0.83860	0.67180	0.60111
4.	gm				
	K as KCl	Total-C = 1.62790	1.51420	1.49400	1.46520
	100 gm soil +	NH <sub>3</sub> -N = 0.81270	0.60610	0.47620	0.37112
	1.0 gm N as	NO <sub>3</sub> -N = 0.01640	0.07140	0.09090	0.13333
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 10	Total-N = 1.02410	0.97690	0.78490	0.73800
	gm				
	K as KCl	Total-C = 1.50240	1.47640	1.45800	1.43450

TABLE 4  
Average Temperature-25°C  
*Light (Unsterile)*

Treatment	Original amounts present in grams per 100 grams of the mixture	Final amounts obtained in grams per 100 grams of the mixture		
		After 15 days	After 30 days	After 60 days
1. 100 gm soil + 1.0 gm N as Urea	NH <sub>3</sub> -N=0.00595 NO <sub>3</sub> -N=0.01987 Total-N=1.24120 Total-C=1.82100	0.06667 0.02500 0.48090 1.58860	0.10010 0.02670 0.34000 1.54200	0.02222 0.04000 0.29750 1.47000
2. 100 gm soil + 1.0 gm N as Urea + 2 gm K as KCl	NH <sub>3</sub> -N=0.00574 NO <sub>3</sub> -N=0.01916 Total-N=1.19655 Total-C=1.75540	0.05333 0.02860 0.56390 1.57020	... ... ... ...	... ... ... ...
3. 100 gm soil + 1.0 gm N as Urea + 5 gm K as KCl	NH <sub>3</sub> -N=0.00544 NO <sub>3</sub> -N=0.01818 Total-N=1.13520 Total-C=1.66540	0.04000 0.03200 0.67840 1.54580	0.08020 0.03333 0.44630 1.50600	0.02500 0.05160 0.35290 1.44000
4. 100 gm soil + 1.0 gm N as Urea × 10 gm K as KCl	NH <sub>3</sub> -N=0.00501 NO <sub>3</sub> -N=0.01674 Total-N=1.04580 Total-C=1.53430	0.03100 0.04710 0.79590 1.52140	0.07270 0.06650 0.57960 1.45800	0.02860 0.07270 0.43700 1.40500

TABLE 5  
Average temperature=25°C  
*Dark (Unsterile)*

1. 100 gm soil + 1.0 gm N as Urea	NH <sub>3</sub> -N=0.00595 NO <sub>3</sub> -N=0.01987 Total-N=1.24120 Total-C=1.82100	0.08340 0.02670 0.54690 1.65600	0.12000 0.02850 0.39260 1.60000	0.03636 0.04450 0.32720 1.51820
2. 100 gm soil + 1.0 gm N as Urea + 2 gm K as KCl	NH <sub>3</sub> -N=0.00574 NO <sub>3</sub> -N=0.01916 Total-N=1.19655 Total-C=1.75540	0.07272 0.03100 0.63570 1.63800	... ... ... ...	... ... ... ...
3. 100 gm soil + 1.0 gm N as Urea + 5 gm K as KCl	NH <sub>3</sub> -N=0.00544 NO <sub>3</sub> -N=0.01818 Total-N=1.13520 Total-C=1.66540	0.05714 0.03480 0.74520 1.61400	0.10020 0.03630 0.49220 1.56000	0.04000 0.05710 0.39160 1.48800
4. 100 gm soil + 1.0 gm N as Urea + 10 gm K as KCl	NH <sub>3</sub> -N=0.00501 NO <sub>3</sub> -N=0.01674 Total-N=1.04580 Total-C=1.53430	0.04450 0.05000 0.86290 1.53040	0.09230 0.06900 0.63690 1.50000	0.04450 0.08010 0.47650 1.45760

It is clear from the foregoing results that there is a considerable loss of nitrogen when ammonium sulphate or urea has been applied to the soil. Again the presence of potassium chloride in the system greatly retard nitrogen loss and greater the concentration of potassium chloride added smaller is the loss of nitrogen. The percentage losses of nitrogen both from ammonium sulphate and urea in presence and absence of different doses of potassium chloride have been calculated and are presented in the following tables:—

TABLE No. 6

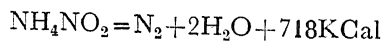
<i>Unsterile</i>		Average Temperature = 25°C					
		Percentage loss of nitrogen from Ammonium sulphate					
Concentration of K added as KCl		After 15 days		After 30 days		After 60 days	
		Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark
1.	nil	55.56	50.45	63.78	58.98	66.80	63.22
2.	2 gm	44.01	38.20	...	...	...	...
3.	5 gm	30.20	24.42	45.32	39.46	49.68	45.83
4.	10 gm	10.77	4.61	28.75	23.36	32.71	27.94

TABLE No. 7

<i>Unsterile</i>		Average Temperature = 25°C					
		Percentage loss of nitrogen from Urea					
Concentration of K added as KCl		After 15 days		After 30 days		After 60 days	
		Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark
1.	nil	61.26	55.94	72.63	68.37	76.03	73.64
2.	2 gm	52.87	46.87	...	...	...	...
3.	5 gm	40.24	34.35	60.68	56.64	68.84	65.50
4.	10 gm	23.89	17.49	44.58	39.10	58.21	54.44

The foregoing experimental results clearly show that when nitrogen rich compounds like ammonium sulphate or urea are added to the soil they undergo slow nitrification leading to a considerable loss of nitrogen but a fair proportion is also converted into nitrate nitrogen. The loss of nitrogen from the soil in sets receiving artificial light is always greater than in those kept in the dark. Again the loss of nitrogen in the case of urea is more pronounced than in the case of ammonium sulphate. This is due to the fact that urea readily changes into ammonium carbonate which slowly decomposes into carbon dioxide and ammonia.

The loss of nitrogen can be explained from the viewpoint that during nitrification of these nitrogenous compounds, an unstable intermediate product ammonium nitrite is formed and this readily decomposes into water and nitrogen gas which escapes and causes the loss of nitrogen according to the following equation :



As the oxidation of these nitrogen rich compounds is more pronounced in light than in the dark, there is always the possibility of greater amounts of formation and decomposition of ammonium nitrite in the sets exposed to light than in those kept in the dark and hence there is always more nitrogen loss found in exposed sets than in covered ones. These results, therefore support the conclusion that

light plays an important part in the process of nitrification and nitrogen loss. Dhar<sup>6,7</sup> has in a number of papers emphasised that the process of nitrification and nitrogen loss may be chiefly photochemical and surface oxidation reaction. Corbet<sup>8</sup>, de Rossi<sup>9</sup> and Sarkaria<sup>10</sup> are in general agreement with Dhar's observations.

Another most important point brought out by these results is that the presence of potassium chloride in the system greatly retards the loss of nitrogen both from ammonium sulphate and urea and increase nitrate formation both in light as well as in the dark. Again the percentage loss of nitrogen decreases and the nitrate formation increases with increasing concentrations of potassium chloride in the system, both from ammonium sulphate and urea.

This is due to the fact that when potassium chloride is present in the system it can react with the unstable substance ammonium nitrite formed in the nitrification of ammonium sulphate and urea and generate nitrite of the alkali metal, which being more stable than ammonium nitrite, remains in the system and can slowly oxidise into nitrate of the alkali metal.

Field trials have shown that the recovery of nitrogen by crops never exceeds 50%, whilst the recovery of phosphate and potash may even go up to 85%. Lohnis and Fred<sup>11</sup> have reported the following recovery in field experiments lasting for four years

Nitrogen	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
7.8 to 46.1%	10.1 to 75.6%	22.4 to 85.1%

It will also be of interest to quote here some of the observations made at Rothamsted<sup>12</sup> on the crop yield per acre on a soil treated with ammonium sulphate containing enough potassium salts and phosphates.

#### EFFECT OF PHOSPHATES AND POTASSIUM SALTS ON THE UTILISATION OF NITRATES BY WHEAT PLANTS

##### BROADBALK

Treatment	Crop yield per acre per annum		Nitrogen recovered in crops lb per acre	Nitrogen present as nitrates in drainage water during Autumn parts per million	Per cent of N in soil	N lost from soil, lb per annum
	Grain bushels	Straw cwt				
Ammonium salts containing 86 lb N + abundant supplies of P and K salts	26.7	30.75	33.5	8.5	0.116	51.0
No P or K salts	16.0	14.75	45.0	17.8	0.106	67.5

These results clearly prove that the nitrogen lost in soils from ammonium sulphate is more where no potassium salts or phosphates have been applied and are in agreement with the results obtained by us.

It is clear, therefore, that an increase in potassium content in the system leads to a less loss of nitrogen from nitrogenous compounds and greater amount of nitrate formation, and this will be profitable from the agricultural point of view. Hence, the presence of these salts should be able to minimise the amount of nitrogenous compounds added to the soil. It will be of interest to point out here that under temperate conditions due to the presence of large amount of humus the addition of ammonium salts leads to the adsorption of the majority of ammonium ions by the humus and the clay particles and, therefore, will not be helpful for plants. If to such a system potassium salts are added, ammonium ions will be displaced by potassium ions from humus and the clay layer and thus will pass into the solution phase to be readily nitrified for the use of crops. In such cases the addition of potassium compounds may intensify the nitrogen effect and show positive interaction between potash and nitrogen as has been occasionally reported for potatoes at Rothamsted. For growing potatoes, beats and perhaps other tubers potash is of great value since these materials require large amount of potash for their growth. It seems, therefore, that in growing tubers potash is not only favourable by its direct action as a plant food material but may also act as an intensifier of nitrogen effect.

#### SUMMARY

1. The majority of nitrogen added to soil in the form of ammonium sulphate or urea is lost from the soil due to the formation and decomposition of the unstable substance ammonium nitrite which readily decomposes into nitrogen gas and water, thus causing the loss of nitrogen. The loss of nitrogen is more in light than in the dark.
2. Potassium chloride added to the soil along with ammonium sulphate retards the loss of nitrogen due to the formation of nitrite of the alkali metal which being more stable than ammonium nitrite remains in the system and can slowly oxidise into nitrate of the alkali metal.

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## EFFECT OF ORGANIC MATTER ON PHOSPHATE AVAILABILITY

By N. R. DHAR and K. L. NAGPAL

*Sheila Dhar Institute of Soil Science, University of Allahabad, Allahabad*

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Numerous investigations extending over the last 80 years have shown that only 10 to 20 per cent. of the applied phosphate is utilised by crops, and the rest is fixed in a form not readily available. In acid soils the added phosphate is fixed as iron and aluminium phosphate and in neutral and alkaline soil it is fixed as insoluble calcium phosphate or even more insoluble apatites. Therefore the availability of the added or the soil phosphate to the plant constitutes a problem of great agricultural importance.

The object of this work is to study the effect of organic matter (which is generally believed to enhance the availability of phosphate) alone and in combination with potassium and calcium salts on the availability of the different phosphates added to the soil. The variations in exchangeable calcium in the soil on the addition of different phosphates have also been studied.

### EXPERIMENTAL PROCEDURE

The soil employed in these investigations was taken from a plot in front of the Chemistry Department Allahabad University.

200 grams of powdered soil after being passed through a sieve of 80 mesh per inch was taken in 250 c.c. beakers. To this soil 0.5 per cent. carbon in the form of well powdered neem leaf was added. Also 0.5 per cent.  $P_2O_5$  in the form of either monocalcium, dicalcium, tricalcium, iron and aluminium phosphates were added to these mixtures. In some cases 0.2 per cent. CaO as  $CaCO_3$  was added while in others 1 per cent. KCl was added. These beakers were placed under a 500 watt electric bulb hung over the table at a distance of  $2\frac{1}{2}$  feet. A similar set of beakers containing the same amounts of materials were placed besides these beakers covered with a thick black cloth. The moisture of the system was maintained at 20 per cent. The contents of the beakers were stirred on every alternate day with a glass rod in order to facilitate the oxidation of the organic matter. The exposure was carried out for a period of six months.

Available phosphate was determined by Dyer's method using 1 per cent. citric acid for extracting the available phosphate. The precipitation was done with a freshly prepared ammonium molybdate solution and the washed precipitate was dissolved in dilute sodium hydroxide solution. Excess of sodium hydroxide was titrated against standard sulphuric acid. Exchangeable calcium was determined by leaching the soil with normal neutral ammonium acetate and the precipitation was carried out by means of ammonium oxalate. All the soil samples were dried before starting the analysis.

### *Soil Analysis*

Total Phosphate	= 0.0841 %	Total Calcium	= 0.9924 %
Available phosphate	= 0.0302 %	Exchangeable Calcium	= 23.1 m.e.

### *Neem Leaf Analysis*

Total Phosphate = 0.138 %

Total Calcium = 1.65 %

200 grams soil + 0.5 % C as Neem leaf.

State	Period of exposure in days	Available phosphate %	Exchangeable calcium m.e.
Exposed	0	0.0302	23.2
	180	0.0318	23.9
Covered	0	0.0302	23.2
	180	0.0310	23.6

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Monocalcium Phosphate

Exposed	0	0.4101	24.6
	180	0.3313	22.4
Covered	0	0.4103	24.4
	180	0.3274	21.8

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Monocalcium Phosphate + 1 % KCl

Exposed	0	0.4092	24.5
	180	0.3104	21.7
Covered	0	0.4092	24.5
	180	0.3078	21.2

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Monocalcium Phosphate + 0.2 %  
CaO as  $CaCO_3$

Exposed	0	0.4103	25.7
	180	0.3436	23.9
Covered	0	0.4101	25.7
	180	0.3369	23.5

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Dicalcium Phosphate

Exposed	0	0.3886	23.8
	180	0.3928	24.7
Covered	0	0.3886	23.8
	180	0.3912	24.3

200 grams soil + 0.5 %  $P_2O_5$  + 0.5 % C + 1 % KCl. as Dicalcium Phosphate

Exposed	0	0.3882	23.6
	180	0.3890	24.2
Covered	0	0.3882	23.7
	180	0.3876	23.9



200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Dicalcium Phosphate + 0.2 % CaO  
as  $CaCO_3$

Exposed	0	0.3882	24.9
	180	0.4065	26.8
Covered	0	0.3879	24.7
	180	0.4032	26.3

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Tricalcium Phosphate

Exposed	0	0.3467	23.6
	180	0.3872	25.7
Covered	0	0.3467	23.4
	180	0.3823	25.2

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Tricalcium Phosphate + 1 % KCl

Exposed	0	0.3462	23.6
	180	0.3811	24.9
Covered	0	0.3462	23.5
	180	0.3781	24.6

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Tricalcium Phosphate + 0.2 %  
CaO as  $CaCO_3$

Exposed	0	0.3464	24.1
	180	0.3954	28.1
Covered	0	0.3464	24.5
	180	0.3913	27.1

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Aluminium Phosphate

Exposed	0	0.3563	23.3
	180	0.3412	21.6
Covered	0	0.3561	23.3
	180	0.3375	21.1

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Aluminium Phosphate + 1 % KCl

Exposed	0	0.3563	23.2
	180	0.3376	20.8
Covered	0	0.3561	23.2
	180	0.3342	20.1

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Aluminium Phosphate + 0.2 %  
CaO as  $CaCO_3$

Exposed	0	0.3561	23.9
	180	0.3888	24.8
Covered	0	0.3561	23.8
	180	0.3558	24.3

200 grams soil + 0.5 % C + 0.5 %  $P_2O_5$  as Iron Phosphate

Exposed	0	0.3531	23.2
	180	0.3401	20.9
Covered	0	0.3527	23.1
	180	0.3386	20.4

200 grams soil + 0.5% C + 0.15%  $P_2O_5$  as Iron Phosphate + 1% KCl

Exposed	0	0.3526	23.2
	180	0.3341	20.2
Covered	0	0.3531	23.1
	180	0.3306	19.7

200 grams soil + 0.5% C + 0.5%  $P_2O_5$  as Iron Phosphate + 0.2% CaO

		as $CaCO_3$	
Exposed	0	0.3528	23.8
	180	0.3552	24.6
Covered	0	0.3528	23.8
	180	0.3531	24.1

200 grams soil + 0.5% C + 0.1% N as Ammonium Sulphate

Exposed	0	0.0302	23.3
	180	0.0341	26.2
Covered	0	0.0302	23.1
	180	0.0324	25.7

200 grams soil + 0.5% C + 0.1% N as Urea

Exposed	0	0.0305	23.2
	180	0.0327	25.8
Covered	0	0.0305	23.1
	180	0.0318	25.4

200 grams soil + 0.5% C + 0.1% N as  $NaNO_3$

Exposed	0	0.0302	23.1
	180	0.0291	22.7
Covered	0	0.0302	23.2
	180	0.0282	22.5

#### DISCUSSION

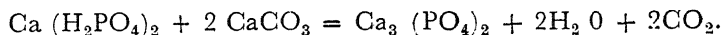
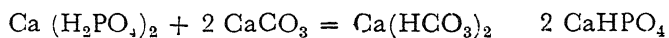
A careful study of the above experimental results shows that the organic matter like neem leaf when mixed with different phosphates in the soil and allowed to oxidize helps in increasing the available phosphate. Other workers like Hester<sup>1</sup>, Baur,<sup>2</sup> Copland and Merkla<sup>3</sup> have also observed that organic matter increased the availability of soil phosphate and of rock phosphate which was added to the soil as a fertilizer. This beneficial effect of organic matter has been attributed to (a) the protective action of organic colloids, (b) the action of  $CO_2$  produced by organic matter decomposition in dissolving certain phosphatic materials (c) formation of organic phosphates which are less firmly fixed up by soils than are inorganic phosphates, (d) mineralisation of organic matter containing phosphates with the resultant release of phosphate for plant use. It may be mentioned here that the tricalcium, dicalcium, iron and aluminium phosphates which have been used in these experiments are partly soluble in 1% citric acid which has been employed for the determination of the available phosphate.

There is a clear increase in the availability of tricalcium phosphate when it is used in conjunction with neem leaf. The carbonic and the small amounts of other organic acids produced in the oxidation and decomposition of organic matter convert the initially unavailable tricalcium phosphate to a more soluble dicalcium

phosphate and very small amounts of monocalcium phosphate resulting in an increase in the amount of available phosphate.

There is only a small increase with dicalcium phosphate. This is because the second dissociation constant of phosphoric acid is only slightly smaller than the dissociation constant of carbonic acid therefore the formation of monocalcium phosphate by the interaction of carbonic acid on dicalcium phosphate is difficult.

With monocalcium phosphate however, there is a decrease in the initially high available phosphate. Dhar has explained it by suggesting that monocalcium phosphate reacts with calcium carbonate forming di and tricalcium phosphates which are less soluble than the monocalcium phosphate.



Iron and aluminium phosphates also have shown a decrease when they are used along with the organic matter. It seems that the carbonic and the other weak acids derived from the oxidation of the organic matter do not much increase the solubility of iron and aluminium phosphates. This is supported by the experiment of B. Lachowicz<sup>4</sup> who has observed that the amount of  $\text{P}_2\text{O}_5$  dissolved from  $\text{FePO}_4$  does not change on saturating distilled water with carbon dioxide but in presence of sodium bicarbonate or sodium acetate, there is appreciable increase of  $\text{P}_2\text{O}_5$  in solution.

When calcium carbonate is used in conjunction with organic matter, the availability of all phosphates except the monocalcium phosphate shows an increase. In the case of monocalcium phosphate the decrease in the available phosphate is a little checked. Other workers like Parker and Tidmore<sup>5</sup>, Salter and Barnes<sup>6</sup>, Halim Damaty and Axley<sup>7</sup> have also obtained similar results. The increase with calcium carbonate is partly accounted by the conversion of some portions of the insoluble iron and aluminium phosphates to relatively more soluble calcium phosphates. Besides the oxidation of the organic matter is also increased by the addition of  $\text{CaCO}_3$  into the system which leads to a greater release of carbonic acid. It is also probable that liming may result in a liberation of the organic phosphorus compounds in the soil through the stimulation of decomposition processes.

The treatment with potassium chloride on the other hand invariably depresses the amount of available phosphate Eriksson<sup>8</sup> reported a reduction in the yield of grain when muriate of potash was applied in combination with superphosphate. Damaty and Axley<sup>9</sup> also observed that KCl decreases the availability of phosphate.

Organic matter when used in combination with artificial nitrogenous fertilizers like ammonium sulphate and urea increase the available phosphate whereas with sodium nitrate there is a decrease. The increase in the availability is due to the acidity produced in the system in the process of nitrification of ammonium sulphate and urea. The nitrous and nitric acids formed in the nitrification process help in making the soil phosphate more available.

The results of exchangeable calcium show the same pattern as that of available phosphate. There is a marked increase in the exchangeable calcium with tricalcium phosphate. There is some increase with dicalcium phosphate also. But there is a decrease with the others.

There results show that there is always more availability in the exposed sets than in the covered ones. This is because of the greater oxidation of the organic matter in exposed set as compared with the covered ones.

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## EFFECT OF PHOSPHATES ON NITROGEN FIXATION

By N. R. DHAR and K. L. NAGPAL

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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In a number of publications from this Laboratory Dhar and co-workers have shown that when different energy producing materials such as glycerol, starch, fats, cellulose, straw, sawdust, leaves, plants, peat, lignite, etc., undergo slow oxidation in air in presence of soil, sand or any other surface, then along with the oxidation of the carbonaceous compounds there is appreciable fixation of atmospheric nitrogen which is always more pronounced in light than in the dark. The object of this work is to study the effect of different phosphate alone and in combination with potassium chloride on the fixation of nitrogen in soil effected by the decomposition of sawdust derived from Teakwood.

### EXPERIMENTAL

The soil used in these investigations was taken from a plot in front of the Sheila Dhar Institute of Soil Science, Allahabad.

200 grams of powdered soil after being passed through a sieve of 80 mesh were taken in 250 c. c. Beakers. To this soil 0.5% carbon in the form of well powdered sawdust was added. Also 0.2%  $P_2O_5$  in the form of either monocalcium, dicalcium, tricalcium, iron and aluminium phosphates were added to these mixtures. In some cases 1% KCl was also added along with the phosphates. These beakers were placed under a 500 Watts electric bulb hung over the table at a distance of 2½ feet. A similar set of beakers containing the same amounts of materials were placed besides these beakers covered with a thick black cloth. The moisture of the system was maintained at 20%. The contents of the beakers were stirred on every alternate day with a glass rod in order to facilitate the oxidation of the organic matter. Carbon, nitrogen and the Azotobacter counts of the soil samples from these beakers were first determined after three months and then again after another three months.

The total carbon present in the samples of the soils was determined by the method described by Robinson, Mclean and Williams.<sup>4</sup> The total nitrogen was determined by salicylic acid reduction method.<sup>5</sup> The Azotobacter counts were determined by Beijerinck's medium. All the soil samples were oven-dried before starting the analysis.

The following are the analyses of the soil and the sawdust which have been used in this work :—

<i>Soil Analysis</i>		<i>Sawdust Analysis</i>	
	%		%
Loss on ignition	= 7.25	Ash	= 10.84
HCl insoluble	= 7.31	Silica	= 4.34
Sesquioxide	= 9.1	Sesquioxide	= 1.589

# Soil Analysis

	%
Fe <sub>2</sub> O <sub>3</sub>	= 3.43
CaO	= 4.31
MgO	= 1.71
K <sub>2</sub> O	= 1.14
P <sub>2</sub> O <sub>5</sub>	= 0.413
Total Carbon	= 1.7453
Total Nitrogen	= 0.2373
Azotobacter	= 2.8 millions per gram of soil

# Sawdust Analysis

	%
CaO	= 1.17
MgO	= 0.6728
K <sub>2</sub> O	= 1.278
P <sub>2</sub> O <sub>5</sub>	= 0.387
Total Carbon	= 45.23
Total Nitrogen	= 0.8371

Period of exposure in days	Total Carbon %	C oxidized %	Total Nitrogen %	Increase in N	Efficiency	Azotobacter millions per gram
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200 grams soil + 0.5% C as Sawdust

*Exposed*

0	2.2241	...	0.2439	...	...	2.8
90	1.9395	0.2846	0.2672	0.0233	81.8	...
180	1.8527	0.3714	0.2737	0.0298	80.2	5.9

*Covered*

0	2.2241	...	0.2439	...	...	2.8
90	2.0507	0.1734	0.2511	0.0072	41.5	...
180	1.9928	0.2313	0.2533	0.0094	40.6	8.1

200 grams soil + 0.5% C + 0.2% P<sub>2</sub>O<sub>5</sub> as Monocalcium Phosphate

*Exposed*

0	2.2243	...	0.2442	...	...	2.8
90	1.9196	0.3047	0.2739	0.0297	97.4	...
180	1.8362	0.3881	0.2813	0.0371	95.5	7.5

*Covered*

0	2.2243	...	0.2442	...	...	2.8
90	2.0442	0.1801	0.2535	0.0093	51.6	...
180	1.9603	0.2640	0.2574	0.0133	50.3	10.6

200 grams soil + 0.5% C + 0.2% P<sub>2</sub>O<sub>5</sub> as Monocalcium Phosphate + 1% KCl

*Exposed*

0	2.2243	...	0.2442	...	...	2.8
90	1.9160	0.3083	0.2765	0.0323	104.8	...
180	1.8282	0.3961	0.2839	0.0397	100.2	8.4

*Covered*

0	2.2243	...	0.2442	...	...	2.8
90	2.0406	0.1837	0.2545	0.0103	56.06	...
180	1.9568	0.2675	0.2589	0.0147	54.9	11.2

200 grams soil + 0.5% C + 0.2% P<sub>2</sub>O<sub>5</sub> as Dicalcium Phosphate

*Exposed*

0	2.2241	...	0.2439	...	...	2.8
90	1.9017	0.3224	0.2786	0.0347	107.6	...
180	1.7957	0.4284	0.2884	0.0445	103.8	8.1

Period of Exposure in days	Total Carbon %	C oxidized %	Total Nitrogen %	Increase in N	Efficiency	Azotobacter millions per gram
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*Covered*

0	2.2241	...	0.2439	...	...	2.8
90	2.0242	0.1999	0.2553	0.0114	57.2	...
180	1.9406	0.2835	0.2592	0.0153	53.6	5.11

200 grams soil + 0.5% C + 0.20%  $P_2O_5$  as Dicalcium Phosphate + 1% KCl

*Exposed*

0	2.2240	...	0.2440	...	...	2.8
90	1.8971	0.3269	0.2817	0.0377	115.3	...
180	1.7208	0.4332	0.2902	0.0480	110.8	9.1

*Covered*

0	2.2440	...	0.2440	...	...	2.8
90	2.0212	0.2028	0.2561	0.0121	59.6	...
180	1.9343	0.2891	0.2603	0.0163	56.3	12.2

200 grams soil + 0.5% C + 0.2%  $P_2O_5$  as Tricalcium Phosphate

*Exposed*

0	2.2234	...	0.2443	...	...	2.8
90	1.8886	0.3348	0.2780	0.0337	100.6	...
180	1.7786	0.4448	0.2878	0.0435	97.6	7.9

*Covered*

0	2.2234	...	0.2443	...	...	2.8
90	2.0159	0.2075	0.2653	0.0110	53.01	...
180	1.9230	0.3004	0.2597	0.0154	51.2	11.3

200 grams soil + 0.5% C + 0.2%  $P_2O_5$  as Tricalcium Phosphate + 1% KCl

*Exposed*

0	2.2239	...	0.2442	...	...	2.8
90	1.8790	0.3449	0.2834	0.0392	113.6	...
180	1.7655	0.4584	0.2936	0.0494	107.7	9.0

*Covered*

0	2.2239	...	0.2442	...	...	2.8
90	2.0070	0.2169	0.2568	0.0126	58.09	...
180	1.9136	0.3103	0.2616	0.0174	56.07	12.0

200 grams soil + 0.5% C + 0.2%  $P_2O_5$  as Iron Phosphate

*Exposed*

0	2.2238	...	0.2441	...	...	2.8
90	1.9103	0.3135	0.2714	0.0273	87.08	...
180	1.7983	0.4255	0.2809	0.0368	86.4	6.6

*Covered*

0	2.2238	...	0.2441	...	...	2.8
90	2.0366	0.1872	0.2528	0.0087	46.4	...
180	1.9423	0.2815	0.2568	0.0127	45.1	10.1

Period of exposure in days	Total Carbon %	C oxidized %	Total Nitrogen %	Increase in N Efficiency	Azotobacter millions per gram	
200 grams soil + 0.5% C + 0.2% P <sub>2</sub> O <sub>5</sub> as Iron Phosphate + 1% KCl						
<i>Exposed</i>						
0	2.2242	...	0.2440	...	...	2.8
90	1.9050	0.3192	0.2732	0.0292	91.4	...
180	1.7939	0.4304	0.2822	0.0382	88.7	7.6
<i>Covered</i>						
0	2.2242	...	0.2440	...	...	2.8
9	2.0322	0.1920	0.2535	0.0095	49.4	...
180	1.9392	0.2850	0.2578	0.0138	48.4	10.8
200 grams soil + 0.5% C + 0.2% P <sub>2</sub> O <sub>5</sub> as Aluminium Phosphate						
<i>Exposed</i>						
0	2.2239	...	0.2441	...	...	2.8
90	1.9160	0.3079	0.2705	0.0264	85.7	...
180	1.7994	0.4245	0.2796	0.0355	83.7	6.3
<i>Covered</i>						
0	2.2239	...	0.2441	...	...	2.8
90	2.0414	0.1825	0.2521	0.0080	43.9	...
180	1.9435	0.2804	0.2559	0.0118	42.08	9.7
200 grams soil + 0.5% C + 0.2% P <sub>2</sub> O <sub>5</sub> as Aluminium Phosphate + 1% KCl						
<i>Exposed</i>						
0	2.2240	...	0.2441	...	...	2.8
90	1.9137	0.3103	0.2718	0.0277	89.3	...
180	1.7954	0.4286	0.2820	0.0379	88.4	7.2
<i>Covered</i>						
0	2.2240	...	0.2441	...	...	2.8
90	2.0.90	0.1850	0.2526	0.0085	45.9	...
180	1.9406	0.2834	0.2567	0.0126	44.4	10.5

#### DISCUSSION

A persual of the foregoing experimental results show that in every case the carbon of the system decreases and the amount of nitrogen increases. The oxidation of the organic matter and the increase in nitrogen is invariably greater in light than in dark as has been reported by the previous workers. The Azotobacter counts however have the reverse tendency—being always more in the covered sets than those exposed in artificial light.

The oxidation of the added energy material *i.e.*, sawdust increases with the addition of all the phosphates. (The phosphates employed in this work as already stated are that of monocalcium, dicalcium, tricalcium, iron and aluminium). Tricalcium phosphate being alkaline causes the highest increase in the oxidation of the organic matter, the rest coming in the following order dicalcium phosphate > monocalcium phosphate > iron phosphate > aluminium phosphate. Prescott also concludes from his experiments with phosphatic fertilizers that



"they are not only concerned in the nutrition of the crop plant but may be found to play an important part in the decomposition of the organic matter added to the soil." On the incorporation of potassium chloride with phosphates, the oxidation of the organic matter increase slightly. It appears that potassium chloride also helps in the oxidation of the organic matter.

The Azotobacter counts also increase with the addition of phosphates but as already stated their number is always greater in dark than in light. If the Azotobacter were the only source for the fixation of nitrogen in the experiments, then in every case the amount of nitrogen should have been greater in dark than in light. But these experimental results indicate that the efficiency (the amount of nitrogen fixed in mgms per gram of carbon oxidized) of nitrogen fixation is invariably higher in light which goes to show Azotobacter is not the sole agency for the fixation of atmospheric nitrogen. Recently Jensen in Denmark, Stockle in Zurich and Lamoigne in Paris have emphasised that Azotobacter does not play a big role in the nitrogen status of the soil.

The above results show that phosphates increase the efficiency of nitrogen fixation. Highest efficiency is obtained with dicalcium phosphate. Monocalcium phosphate which is distinctly acidic is not as effective in the increase of nitrogen as di and tricalcium phosphates. The increase in the case of phosphates of iron and aluminium is smaller than with mono, di and tricalcium phosphates, both of these materials having almost an equal effect. The increase in the fixation of nitrogen by the addition of phosphates may be explained by their increased oxidation of the added organic matter which leads to more nitrogen fixation. Dhar has suggested that phosphates may also form stabler kinds of phospho-proteins which seem to be more resistant to oxidation than the ordinary protein and thus may be responsible for checking the loss of nitrogen.

When potassium chloride is also added to the soil along with sawdust and phosphates the efficiency of nitrogen fixation increases. In all the cases the highest efficiency of nitrogen fixation is obtained when a mixture of phosphate and potassium chloride is incorporated with the organic matter. It seems that the nitrate formed in the nitrification process combines with potassium forming potassium nitrate which is a stabler compound thus checking the loss of nitrogen.

Thus it may be concluded that phosphates in combination with the organic matter can play a great part in building up permanent soil fertility by fixing large amounts of atmospheric nitrogen. Addition of potassium chloride helps process.

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# STUDIES ON AVAILABLE PHOSPHATE AND EXCHANGABLE CALCIUM IN SOIL

By. N. R. DHAR and PRITAM SINGH

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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The phosphate problem is of primary agricultural importance. A soil rich in insoluble phosphates is of little use for a good crop production. It is the available phosphate status of a soil that matters. When a soluble phosphate fertilizer is added to the soil, a part of it gets adsorbed by the colloidal complexes, in which form it is still partially available to plants. The excess phosphate enters into combination with the calcium of the soil forming tricalcium phosphate and some dicalcium phosphate in alkaline soil. In acid soils, however, it forms mostly iron and aluminium phosphates which are less readily soluble than calcium phosphate.

It appears that the study of the problem of phosphate fixation has to be continued in various directions. There are scientists<sup>1</sup> who remark that natural soils contain so many substances capable of fixing phosphates that it is hopeless to unravel the mechanism of fixation of phosphate by the study of such complex systems, while others<sup>2</sup> are of the opinion that so called "phosphate fixation" is not a serious problem and that phosphate fixation should not be used as an argument against the use of soluble phosphate fertilizers. The present study of the authors is yet another effort towards throwing light on this difficult problem.

## EXPERIMENTAL

Two hundred grams of soil were taken in shallow enamelled dishes of 9" diameter. The soil was perviously well powdered and passed through a 80 mesh sieve. 0.5% carbon in the form of well powdered, air dried, whole lucerne plant was added. Monocalcium phosphate, diphosphate, tricalcium phosphate, iron phosphate, and aluminium phosphates were added in the dose of 0.2%, 1% potassium chloride and 0.2% CaO as calcium carbonate were also added. These dishes were exposed to the light of a 500Watt electric bulb, kept at a distance of two feet from the dishes. Another similar set, covered with thick black cloth was kept besides the exposed dishes. The moisture was kept at 20% throughout. The material in the dishes was stirred with a glass rod on alternate days. Samples were taken after six months. They were oven-dried and analysed for exchangable calcium and available phosphate. Exchangable calcium was determined by the standard ammonium acetate (pH-7) method while available phosphate by Dyer's method using 1% citric acid solution.

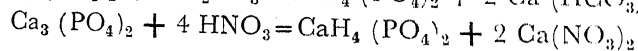
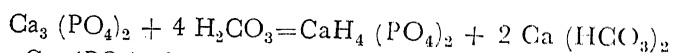
Total $P_2O_5$ in the soil used in these experiments	= 0.0745%.
Available	" " " " " " = 0.0293%.
Total Calcium	" " " " " " = 0.9834%.
Exchangable Calcium	" " " " " " = 22.6 m. c.

Time in days	<i>Light</i>		<i>Dark</i>	
	Available $P_2O_5$	Exch. Ca	Available $P_2O_5$	Exch. Ca
200 gms of soil + 0.5% Carbon as lucerne				
0	0.0293	22.6	0.0293	22.6
180	0.0320	23.4	0.0316	23.2
200 gms of soil + 0.5% Carbon as lucerne + 0.2% CaO as $CaCO_3$				
0	0.0294	22.6	0.0294	22.6
180	0.0372	22.6	0.0359	26.2
200 gms of soil + 0.5% Carbon + 0.2% Monophosphate + 0.2% CaO as $CaCO_3$				
0	0.1984	25.9	0.1984	25.9
180	0.1743	24.4	0.1728	24.2
200 gms of soil + 0.5% Carbon + 0.2% Monocalcium phosphate				
0	0.1980	25.4	0.1980	25.4
180	0.1692	21.1	0.1671	21.0
200 gms of Soil + 0.5% Carbon + 0.2% Monocalcium phosphate + 1% KCl				
0	0.1981	25.6	0.1981	25.6
180	0.1581	19.8	0.1572	19.6
200 gms of soil + 0.5% Carbon + 0.2% Dicalcium phosphate + 0.2% CaO as $CaCO_3$				
0	0.1892	24.8	0.1892	24.8
180	0.2008	28.9	0.1981	28.4
200 gms of soil + 0.5% Carbon + 0.2% $CaHPO_4$				
0	0.1896	24.2	0.1896	24.2
180	0.1922	25.9	0.1911	25.6
200 gms of soil + 0.5% Carbon + 0.2% $CaHPO_4$ + 1% KCl				
0	0.1899	24.2	0.1090	24.2
180	0.1910	25.4	0.1891	25.1
200 gms of soil + 0.5% Carbon as lucerne + 0.2 % Tricalcium phosphate + 0.2% CaO as $CaCO_3$				
0	0.1062	23.6	0.1062	23.0
180	0.1343	29.2	0.1318	28.7
200 gms of soil + 0.5% Carbon as lucerne + 0.2% Tricalcium phosphate				
0	0.1058	23.0	0.1058	23.0
180	0.1140	28.2	0.1113	26.7
200 gms of soil + 0.5% Carbon + 0.2% Tricalcium phosphate + 1% KCl				
0	0.1072	23.0	0.1072	23.0
180	0.1108	27.0	0.1084	27.0
200 gms of soil + 0.5% Carbon + 0.2% $AlPO_4$ + 0.2% CaO as $CaCO_3$				
0	0.0940	22.8	0.0942	23.8
180	0.1034	26.2	0.1002	26.1

Time in days	<i>Light</i>		<i>Dark</i>	
	Available P <sub>2</sub> O <sub>5</sub>	Exch. Ca	Available P <sub>2</sub> O <sub>5</sub>	Exch. Ca
200 gms of soil + 0.5% Carbon + 0.2% AlPO <sub>4</sub>				
0	0.0942	22.6	0.0942	22.6
180	0.0860	21.8	0.0848	21.8
200 gms of soil + 0.5% Carbon + 0.2% AlPO <sub>4</sub> + 1% KCl				
0	0.0943	22.6	0.0943	22.6
180	0.0846	21.8	0.0844	21.8
200 gms of soil + 0.5% C + 0.2% FePO <sub>4</sub> + 0.2% CaO as CaCO <sub>3</sub>				
0	0.0935	22.6	0.0935	22.2
180	0.1009	26.6	0.1001	26.2
200 gms of soil + 0.5% Carbon + 0.2% FePO <sub>4</sub>				
0	0.0922	22.7	0.0922	22.6
180	0.0852	22.0	0.0848	22.0
200 gms of soil + 0.5% Carbon + 0.2% FePO <sub>4</sub> + 1% KCl				
0	0.0924	22.6	0.0924	22.6
180	0.0841	22.0	0.0827	22.0
200 gms of soil + 0.5% Carbon as lucerne + 0.1% Nitrogen as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>				
0	0.0294	22.6	0.0294	22.6
120	0.0352	27.8	0.0329	26.9
200 gms of soil + 0.5% Carbon + 0.1% Nitrogen as Urea.				
0	0.0294	22.6	0.0296	22.6
120	0.0341	27.6	0.0329	26.9
200 gms of soil + 0.5% Carbon + 0.1% Nitrogen as Sodium nitrate				
0	0.0294	22.6	0.0294	22.6
120	0.0280	23.1	0.0280	22.4

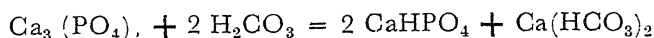
#### DISCUSSION

An analysis of the aforementioned results show that organic matter in the form of lucerne plant when added to the soil and allowed to be oxidised for six months, increases the availability of phosphorus. Jensen<sup>3</sup> also showed that addition of organic matter to a soil increases the solubility of both lime and phosphoric acid 30 to 100%. This is generally held to be due to the conversion of tricalcium phosphate present in the soil to monocalcium phosphate state, by the action of carbonic acid and nitric acid produced in the decomposition, and nitrification of organic matter.<sup>4</sup>



But according to Dhar,<sup>5</sup> the formation of dicalcium phosphate by the action of carbonic acid on tricalcium phosphate is certainly easier than the

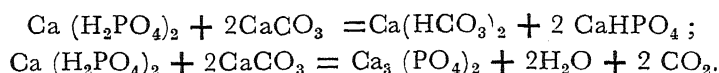
formation of the readily soluble monocalcium phosphate as the dissociation constant of phosphoric acid is much higher than the dissociation constant of carbonic acid.



When calcium carbonate is added along with the plant material, the availability of phosphate increases. This may be partly due to the increased oxidation of organic matter, which is brought about by the introduction of alkalinity of the calcium carbonate into the system. Moreover, since liming increases the calcium content of the soil, the phosphate that is present in the soil as iron and aluminium phosphate is converted in part to the more available calcium phosphate. This is especially true of acid soils containing considerable quantities of iron and aluminium phosphate.

In our experiments with iron and aluminium phosphates which are initially partly soluble in 1% citric acid, we have observed a decrease in the availability when they are mixed into soil along with organic matter. It appears that some of the initially 1% citric acid soluble iron and aluminium phosphates may be converted into the more insoluble iron and aluminium phosphate. Moreover this decrease in the availability of phosphate clearly demonstrate that carbonic acid is not capable of increasing the solubility of iron and aluminium phosphates. This is supported by the experiments of B. Lachourez<sup>6</sup> who has observed that the amount of  $\text{P}_2\text{O}_5$  dissolved from ferric phosphate does not change on saturating distilled water with carbon dioxide but in presence of sodium bicarbonate and sodium acetate, there is appreciable increase of  $\text{P}_2\text{O}_5$  in solution. In our experiments also, in presence of calcium carbonate, the availability of the phosphate appreciably increases in the system containing organic matter and phosphates of iron and aluminium.

Addition of mono-phosphate entails a decrease in the availability with lapse of time. Dhar has suggested the following mechanism for this :



With dicalcium phosphate there is little increase in the availability of phosphate. Dhar has explained it by observing that because the second dissociation constant  $\text{H}_3\text{PO}_4$  is only slightly lower than the dissociation constant of carbonic acid, the formation of monocalcium phosphate by the interaction of carbonic acid on diphosphate and tricalcium phosphate is difficult. But carbonic acid can act upon tricalcium phosphate to give diphosphate and this easily explain this increase of availability of phosphate observed by us on addition of tricalcium phosphate. Baur,<sup>7</sup> Ramaswami Sivan,<sup>8</sup> McGeorge<sup>9</sup> and Rahn<sup>10</sup> have all concluded that organic matter is valuable in increasing the availability of phosphate manure.

When the various phosphates were added fortified with calcium carbonate, there was an overall increase in availability except in the case of monocalcium phosphate, where the initial decrease in the availability was lessened. Increasing exchangeable calcium of the soil tended to increase phosphate availability. Parker, Tidmore<sup>11</sup> and Salter and Barnes<sup>12</sup> have also made similar observations.

Application of potassium along with the various phosphates, on the other hand decreased the availability of phosphate, practically in all cases. Singh and

Nijawan<sup>13</sup> observed a decrease in the availability of phosphorus by the presence of potassium in the soil's exchange complex. Halim el Damaty and Axley<sup>14</sup> have also supported this view.

In case of ammonium sulphate and urea, there was a slight increase in the availability of phosphate. This is perhaps due to the acidity that accompanies the addition of these fertilizers to the soil.<sup>15</sup> Sodium nitrate on the other hand brought about a slight decrease in the availability. Lyon, Fippin and Buckman<sup>16</sup> have expressed similar views in this connection.

The authors results of exchangeable calcium estimations reveal that there is a corresponding increase and decrease of exchangeable calcium when compared to available phosphate results. There seems to be a close relationship between the exchangeable calcium and the available phosphate content of the soil under investigation. Generally the increase of exchangeable calcium is not so marked as that of available phosphate but in case of ammonium sulphate and urea, it is quite appreciable. This is perhaps due to the formation of soluble calcium nitrate when ammonium salts get nitrified in soil. This marked increase in exchangeable calcium can also be attributed to the fact that ammonium ion introduced into soil as ammonium sulphate or urea replaces the calcium of the exchange complex.

Invariably there is more availability in exposed sets than in covered sets, though the difference is slight. This is due to the fact that in exposed sets there is more oxidation and so greater liberation of carbonic acid than in covered sets.

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# RETARDING EFFECT OF WHEAT STRAW ON NITROGEN LOSS FROM SOILS ON THE ADDITION OF AMMONIUM SULPHATE

By N. R. DHAR and D. P. GAUR

*Department of Chemistry, University Allahabad*

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Nitrogen being a substance of very elusive nature, the nitrogen fixed or added as manure does not remain for a long time in the soil under ordinary circumstances. As a matter of fact in the transformation of nitrogen, which concerns the fate of nitrogen from its initial fixation into the soil to its final liberation into the atmosphere, involving a series of reactions like nitrogen fixation, protein decomposition, ammonification, nitrification, denitrification, those reactions that involve the fixation of nitrogen and its loss in the gaseous form are easily the most important from the practical point of view.

Evidence has been gathered by Lipman and Blair,<sup>1</sup> Russell and Richards<sup>2</sup> and numerous other workers to show that nitrogen in the gaseous state is lost from soils. Shutt<sup>3</sup>, Snyder,<sup>4</sup> Swanson<sup>5</sup> and Gainey,<sup>6</sup> Bracken and Greaves<sup>7</sup> have found from experiments that only one-third of the nitrogen was recovered in the crop.

The problem of nitrogen loss involves a much more serious consideration in as much as most of the nitrogenous fertilizers given as top dressing to the fields do not improve the nitrogen status of the soils.

The following experimental results throw light on the mechanism of nitrogen loss from the soils with ammonium sulphate, when it is added to soils. The effectiveness of wheat straw in retarding this loss of nitrogen from soils has also been studied.

## EXPERIMENTAL PROCEDURE

300 grams of well-dried, powdered (sieved through a 50 mesh sieve) soil or sand was taken in shallow enamelled dishes and ammonium sulphate (so as to raise the nitrogen content by 0.05%) was added both with and without well powdered, sieved (through a 50 mesh sieve) and well-dried wheat straw (1% and 2%). The initial total carbon and total, ammoniacal, and nitric nitrogen percentages were calculated from the amounts of the substances in the dishes. Two sets of each mixture were taken one was kept exposed to artificial light from an electrical bulb (160 W-230V) whilst the other was kept covered with a thick black cloth by the side of the exposed one. The mixture were daily stirred, the moisture being maintained at nearly 20% by adding water daily to the exposed and on alternate days to the covered sets. The samples were taken out of the mixtures at intervals and analysed for total carbon, total ammoniacal and nitric nitrogen according

to the standard methods of analysis. Mean temperature was recorded during the exposure. The results of analysts are recorded in the following pages :—

#### ANALYSIS OF WHEAT STRAW

	%		%
Moisture	... 4.32	Total carbon	... 30.8
Loss on ignition	... 91.8	Total nitrogen	... 0.5602
Ash	... 8.69		
SiO <sub>2</sub>	... 7.24		
CaO	... 0.230		
K <sub>2</sub> O	... 0.697		
MgO	... 0.092		
P <sub>2</sub> O <sub>5</sub>	... 0.066		

#### EXPERIMENTS WITH SOILS

Analysis of soil (on oven dry basis)

	%
Loss on ignition	... 3.87
HCl insoluble	... 81.65
Sesquioxides	... 11.18
Fe <sub>2</sub> O <sub>3</sub>	... 4.12
CaO	... 1.157
MgO	... 0.992
K <sub>2</sub> O	... 0.989
P <sub>2</sub> O <sub>5</sub>	... 0.079
Total carbon	... 0.4347
Total nitrogen	... 0.0470
NH <sub>3</sub> —N	... 0.0039
NO <sub>3</sub> —N	... 0.0015
Azotobacter count	... 2.8 millions/gm. dry soil.
Pore space	... 49.9
pH	... 7.5

TABLE No. 1

Temperature range—26.5°C to 32°C

300 gms soil + 0.7075 gms. Ammonium sulphate (0.05% N)

Days	Total carbon in 100 gms soil.	Total nitrogen in 100 gms soil	Loss% on added nitrogen	NH <sub>3</sub> —N in 100 gms soil	NO <sub>3</sub> —N in 100 gms soil
<i>Exposed—Unsterile</i>					
0	0.4347	0.0070	—	0.0515	0.0039
15	0.4338	0.0863	21.4	0.0394	0.0049
30	0.4330	0.0787	36.6	0.0308	0.0058
60	0.4315	0.0667	60.6	0.0175	0.0067
<i>Covered—Unsterile</i>					
0	0.4347	0.0970	—	0.0515	0.0039
15	0.4339	0.0894	15.2	0.0427	0.0046
30	0.4331	0.0845	25.0	0.0373	0.0050
60	0.4320	0.0758	42.4	0.0279	0.0055



TABLE No. 2

300 gms soil + 0.7075 gms Ammonium Sulphate + 3 gms straw

Days	Total carbon in 100 gms soil	Total nitrogen in 100 gms soil	Loss% on added nitrogen	NH <sub>3</sub> -N in 100 gms soil	NO <sub>3</sub> -N in 100 gms soil
<i>Exposed—Unsterile</i>					
0	0.8327	0.1026	—	0.0515	0.0039
15	0.8207	0.0975	10.2	0.0456	0.0045
30	0.8067	0.0943	16.6	0.0414	0.0057
60	0.7765	0.0877	29.8	0.0334	0.0069
<i>Covered—Unsterile</i>					
0	0.8327	0.1026	—	0.0515	0.0039
15	0.8244	0.0993	6.6	0.0476	0.0042
30	0.8142	0.0974	10.4	0.0449	0.0049
60	0.8001	0.0933	18.6	0.0403	0.0056

TABLE No. 3

*Exposed—Unsterile*

300 gms soil + 0.7075 gms Ammonium sulphate + 3 gms Straw

0	1.2307	0.1002	—	0.0515	0.0039
15	1.2152	0.1039	8.6	0.0464	0.0045
30	1.1976	0.1011	14.2	0.0425	0.0056
60	1.1685	0.0959	24.6	0.0358	0.0070
<i>Covered—Unsterile</i>					
0	1.2307	0.1082	—	0.0515	0.0039
15	1.2205	0.1054	5.6	0.0481	0.0042
30	1.2089	0.1038	8.8	0.0462	0.0049
60	1.1911	1.1007	15.0	0.0414	0.0058

*Experiments with sand*

Analysis of the sand (on oven dry basis)

	%		%
Loss on ignition	0.80	Total carbon	0.0824
HCl insoluble	92.11	Total nitrogen	0.0084
Sesquioxides	2.34	NH <sub>3</sub> -N	0.0002
Fe <sub>2</sub> O <sub>3</sub>	1.18	NO <sub>3</sub> -N	0.0006
MgO	0.368	Azotobacter count	0.12 million/gm dry sand
CaO	2.416	Pore-space	42.7%
K <sub>2</sub> O	0.987	pH	8.0
P <sub>2</sub> O <sub>5</sub>	0.044		

300 gms sand + 1.4150 gms. ammonium sulphate (0.05%N)

Temperature range 27°C to 31.5°C

TABLE 4

Days	Exposed—Unsterile				
	Total carbon in 100 gms sand	Total nitrogen in 100 gms sand	Loss% in added nitrogen	NH <sub>3</sub> —N in 100 gms sand	NO <sub>3</sub> —N in 100 gms sand
0	0.0824	0.0585	...	0.0502	0.0006
15	0.0820	0.0449	13.5	0.0362	0.0009
30	0.0822	0.0365	21.9	0.0275	0.0013
60	0.0822	0.0219	36.5	0.0124	0.0015

## Covered—Unsterile

0	0.0824	0.0584	...	0.0502	0.0006
15	0.0822	0.0488	9.6	0.0401	0.0008
30	0.0818	0.0427	15.7	0.0340	0.0010
60	0.0820	0.0312	27.2	0.0220	0.0013

300 gms sand + 0.7075 gm Ammonium sulphate + 3 gms Straw

TABLE 5

Exposed—Unsterile					
0	0.4804	0.0640	...	0.0502	0.0006
15	0.4657	0.0581	11.8	0.0441	0.0008
30	0.4523	0.0545	19.0	0.0416	0.0011
60	0.4196	0.0485	31.0	0.0335	0.0018

## Covered—Unsterile

0	0.4804	0.0640	...	0.0502	0.0006
15	0.4707	0.0604	7.2	0.0464	0.0008
30	0.4588	0.0580	12.0	0.0439	0.0010
60	0.4370	0.0537	20.6	0.0387	0.0014

300 gms sand + 0.7075 gm Ammonium sulphate + 6 gms Straw

TABLE 6

Exposed—Unsterile					
0	0.8784	0.0696	...	0.0502	0.0006
15	0.8586	0.0645	10.2	0.0448	0.0008
30	0.8444	0.0607	17.0	0.0409	0.0013
60	0.8051	0.0553	28.6	0.0348	0.0020

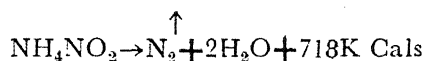
## Covered—Unsterile

0	0.8784	0.0696	...	0.0502	0.0006
15	0.8629	0.0661	7.0	0.0464	0.0007
30	0.8486	0.0636	12.0	0.0434	0.0009
60	0.8282	0.0597	19.8	0.0391	0.0015

## DISCUSSION

From a careful persual of the foregoing results, it becomes quite clear that a major part of nitrogen added as ammonium sulphate to soil or sand is lost without any benefit to soil. The loss is greater in the sets exposed to light than in those kept in dark. Also that increase of nitric nitrogen is more in the sets exposed to light than in those kept in the dark and it gradually increases with the lapse of time. The rate of loss of nitrogen is rapid in the beginning but slows down with the lapse of time. Addition of finely powdered straw markedly checks the loss of nitrogen from the system both in the exposed and the covered sets. The greater the dose of straw, the better is the retarding influence on the loss of nitrogen. It has also been observed that the straw gradually oxidizes and the carbon content of the system decreases.

The most plausible explanation of this type of loss of nitrogen is the formation of the unstable compound, ammonium nitrite, during the nitrification of ammonium salts. It transpires as the ammonium ion gradually undergoes oxidation, the first product of oxidation are nitrite ions, which finally get oxidized to nitrate, thus there is a possibility of ammonium and nitrite ions existing together in the system. These ions may combine to produce the unstable compound, ammonium nitrite, which readily breaks up to give gaseous nitrogen and water, thus :



The gaseous nitrogen so formed escapes out of the system thus causing loss of nitrogen. Thus for this type of denitrification, soil aeration is an essential condition. It is well-known that ammonium salts oxidize more in light than in dark, which explains the greater loss of nitrogen in the exposed sets.

The fact that light is detrimental to bacteria and hence greater number of bacteria are present in the covered sets goes to show that the nitrification and hence the loss is not due to bacterial action as generally believed, because had it been so, the loss should have been greater in the covered sets than in those exposed to light.

The rate of nitrification is observed to slow down with the lapse of time. It is natural and is easily explained on the basis of the "law of mass action."

Another interesting point, which has been brought about in these investigations, is the retarding effect of straw on the nitrogen loss. It has been observed that when the straw is mixed along with ammonium sulphate the loss is markedly checked and the retarding influence becomes more prominent as the dose of straw is increased. It seems that straw, which is carbonaceous material, checks the oxidation of ammonium salts. It is well-known that carbohydrates preserve body proteins from undergoing ammonification. It seems, therefore, that straw also behaves in a similar manner in soils and preserves their nitrogen from being oxidized and thus lost. In fact any carbonaceous matter will behave in a similar manner. Moreover, the straw undergoes oxidation and thus also fixes nitrogen.

Thus, it will be quite clear that importance of organic matter or a mixture of ammonium salts and carbonaceous matter lies in the fact that firstly the soil texture is improved by the colloids added with organic manures, secondly the

carbonaceous matter acts as an agent for the preservation of nitrogenous compounds in the soil by behaving as a negative catalyst and and thirdly it enriches the soil by fixation of nitrogen due to the energy liberated in its oxidation.

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## FIXATION OF ATMOSPHERIC NITROGEN IN SAGAR SOILS

### Part I—With Phosphates Only

By O. N. TRIPATHI and S. N. BANERJI

*Department of Chemistry, University of Sagar*

(Received on 20th November, 1954)

Phosphates play a remarkable role in fixation of nitrogen in the soil, in addition to being assimilated by the growing plants and by the bacterial population of the soil. Phosphate poor soils have generally not been rich in nitrogen fixed from atmosphere. Fixation of nitrogen by *Azotobacter* is found to have been increased by addition of phosphates to the soil. It has also been observed by some workers that application of phosphatic fertilizers, within limits, can be substituted for nitrogen fertilizers. E. Bear<sup>1</sup> says: "Their use stimulates nitrogen-fixing bacteria both non-symbiotic and those associated with legumes to greater activity . . . . The value of phosphatic fertilizers to stimulate nitrogen fixation is even greater for annual or biennial legumes. Excessive fixation occurs if heavy application of phosphates are made in advance of seeding."

It is also well-known that all natural phosphatic manures, especially from bones, essentially contain nitrogen, showing thereby that phosphates, by some process or the other, combine with nitrogen. Bones<sup>2</sup> contain nitrogen and phosphorous in various forms, as shown in table I. (The figures show the percentage composition).

TABLE I

	Nitrogen	P <sub>2</sub> O <sub>5</sub>
Raw Bones	5	22
Bone Meal	3.5 to 4.5	20 to 25
Steamed Bone flour	0.8	27.5 to 30
Dissolved Bone	3	15 to 16
Meat Bone Meal	4 to 8	9 to 18

Synthetic phosphatic fertilizers also have been found to absorb ammonia and improve crop yields. E. Russell<sup>3</sup> writes: "This happens when super-phosphate is mixed with farmyard manure, a practice which can reduce loss of ammonia from manure heaps, if properly used." It has been found that the addition of anhydrous ammonia to super-phosphate increases its nitrogen content to 3 to 4 %. On the other hand, it has also been observed<sup>4</sup> that the use of ammonium salts renders phosphates much more profitable to growing plants. In experiments performed at Rothamsted Experimental Station,<sup>5</sup> a mixture of ammonium and phosphatic fertilizers has shown better results than the two, when used separately.

It is clear, therefore, that phosphatic fertilizers possess the power of furnishing nitrogen to the plants, either by associating itself with the manurial nitrogen or

directly fixing it from atmosphere. Dhar and co-workers<sup>6</sup> have shown that in the soils, having a  $P_2O_5$  content of 0.075 % the efficiency of nitrogen fixation is much smaller than in soils having a phosphate content of 0.33 to 0.41 %.

Jules Joffre,<sup>7</sup> in 1902, claimed that the soils of Pyrenese fix nitrogen in presence of phosphates. In his own words : "The small amounts (0.14 %) of nitrogen in phosphates from Pyrenese was found to be of no Agricultural value." Recently, Dhar<sup>6</sup> has also claimed that in sand as medium, the efficiency of fixation in light is 200 mgm of nitrogen per gm of carbon oxidized in presence of phosphate and other energy materials. The experimental values show that there is very little to choose between the legume manuring and carbohydrate manuring, specially in lime and phosphate rich soils. In presence of phosphates of calcium, in cold countries, oxidation of carbohydrates is sufficiently intensified. His results are supported by a set of experiments, performed both in light and in darkness, where he found that in light, nitrogen fixation, in presence of calcium and phosphate is of the order of 150-75 mg. in unsterile condition and of the order of 84-65 mgs in sterile conditions. Similarly, in dark experiments, the results varying from 80-35 mgs under unsterile condition and 43-82 under sterile conditions were obtained. "Hence we have come to the important conclusion that the nitrogen status of the soil is intimately connected with its phosphorous status and that phosphates cannot act as only food for plants, micro-organisms and buffers in controlling the pH of the soil, but can also help in fixation of atmospheric nitrogen by oxidation of energy materials."<sup>6</sup>

#### EXPERIMENTAL

Three sets of the Sagar soil, each containing 1, 3, and 5 gs of the solution of the phosphate of sodium per pound of the soil, were regularly exposed to sunlight in glass jars. The moisture content of the soil was kept constant. The sets were periodically analysed. The mean temperature during exposures was 42°C.

Nitrogen was determined by the standard Kjeldahl's method.<sup>8</sup> Organic carbon was estimated by Black and Walkley's method,<sup>9</sup> by digesting soil with chromic acid and titrating excess of unused acid by standard ferrous sulphate solution.

The phosphate used was B. D. H. disodium hydrogen phosphate. Analytical results of the phosphate is given below in table II.

TABLE II

		%
Chloride	...	0.006
Sulphate	...	0.030
Heavy metals (Pb)	...	0.003
Iron (Fe)	...	0.0015
As <sub>2</sub> O <sub>3</sub>	...	0.0006
Moisture	...	0.2
Nitrogen	...	...

TABLE III  
ANALYSIS OF SOIL

	%
Total nitrogen	0.0742
Organic carbon	1.142
Ammoniacal nitrogen	0.0019
Nitrate nitrogen	0.00981
CaO	3.0126
MgO	0.2530
K <sub>2</sub> O	0.9852
P <sub>2</sub> O <sub>5</sub>	0.3895

TABLE IV

	Total Nitrogen		
	1 g	3 gs	5 gs
Concentration of phosphate per lb of soil			
Percentage of nitrogen in the soil before phosphate is mixed.	0.0742	...	...
Percentage of nitrogen in soil after exposing for :			
15 days	0.0966	0.1138	0.1174
1 month	0.0982	0.1179	0.1211
2 months	0.0982	0.1186	0.1213
3 "	0.0982	0.1183	0.1217
4 "	0.0981	0.1195	0.1215

TATLE V

	Organic Carbon		
	1 g	3 gs	5 gs
Concentration of the phosphate per lb of soil			
Percentage of organic carbon in the soil before phosphate is mixed.	1.142	...	...
Percentage of organic carbon in the soil after exposing for :			
15 days	0.9964	0.8846	0.8612
1 month	0.9860	0.8580	0.8372
2 months	0.9860	0.8534	0.8359
3 "	0.9795	0.8554	0.8333
4 "	0.9587	0.8476	0.8331

TABLE VI  
Change in Nitrogen content

Concentration of the phosphate per lb of the soil	1 gm	3 gs	5 gs
Percentage of nitrogen in the soil before phosphate in mixed	0.0742	...	...
Percentage of change in nitrogen contents of the soil after exposing for :			
15 days	0.0240	0.0396	0.0432
1 month	0.0240	0.0437	0.0469
2 months	0.0241	0.0444	0.0471
3    ,,	0.0241	0.0441	0.0475
4    ,,	0.0239	0.0453	0.0473

TABLE VII  
Carbon oxidized

Mean temperature 42°C			
Concentration of phosphate per lb of the soil	1 g	3 gs	5 gs
Percentage of organic carbon in the soil before phosphate is mixed	1.142	...	...
Percentage of carbon oxidised in the soil after exposing for :			
15 days	0.1456	0.2574	0.2808
1 month	0.1560	0.2841	0.3049
2 months	0.1563	0.2886	0.3062
3    ,,	0.1566	0.2807	0.3088
4    ,,	0.1853	0.2945	0.3089
Efficiency :	129.5	158.8	153.9

#### DISCUSSION

The mechanism of the reactions involved in the process may be explained as follows :—

During the process of photo-synthesis in plants, decomposition of water molecules takes place by absorption of light energy.

Thus : 
$$\text{H}_2\text{O} - 112 \text{ Cal} = \text{H} + \text{OH}$$

This hydrogen thus formed reacts with carbonic acid, absorbed on leaves, and reduces it to formaldehyde. The same explanation can be forwarded for the



process of nitrogen fixation in soils *i.e.*, decomposition of water in H and OH by absorption of energy obtained from oxidation of carbohydrates, celluloses, lignin, fats, coals, etc. When this reaction is taking place in light, whether artificial or sunlight, light energy is absorbed by the system and utilised in increasing the nitrogen fixation. The same mechanism may be applied in symbiotic nitrogen fixation also.

Further, for fixing 14 gms of nitrogen and forming ammonia by the interaction of nitrogen and hydrogen formed in the decomposition of water, 336 KCal are needed. Hence, from the oxidation of a gram molecule of glucose



Therefore,  $\frac{14 \times 676}{336 \times 72}$  gm of nitrogen can be fixed under ideal conditions.

In other words 0.39 g of nitrogen should be fixed per gm of carbon oxidized. In our experiments in Sagar soils rich in sodium phosphates, fixation of 130 mgm of nitrogen with 1 gm of phosphate per lb of soil and 154 mgm of nitrogen with 3 and 5 gms of phosphates per lb of the soil has been observed, per gm of carbon oxidized (*vide* last column of table VII). Hence the efficiency of nitrogen fixation in the soil is  $\frac{130}{390} \times 100 = 33.3\%$  with 1 gm of phosphate per lb of soil

and  $\frac{145}{390} \times 100 = 39.5\%$  with 3 and 5 gms. of phosphate in each lb of the soil. Thus

our results show that (1) the fixation of nitrogen by the phosphate is maximum when it is applied in a concentration of 3 gs per lb of Sagar soil, and (2) the maximum efficiency of fixation of nitrogen per gm of carbon oxidized is 39.5% under the laboratory conditions.

Our results are in close agreement with those of Dhar<sup>6</sup> who obtained 38% of the efficiency in nitrogen fixation by 2.5 to 1 gm of the phosphate of calcium and exposed to 60 watt bulb for 80 hours.

The fundamental chemical changes involved in all types of nitrogen fixation are identical. In symbiotic fixation also the Rhizobia, containing haemoglobin, may be able to oxidize the carbohydrates synthesized by host plant very rapidly, creating an efficient nitrogen fixation process which certainly requires an influx of energy. The phosphate, therefore, seems to increase efficiency of nitrogen fixation in all types of nitrogen fixation processes in which ammonia formation is an important step. It may be concluded that nitrogen status of all soils of all countries, not under legumes, appears to be chiefly due to surface and photo-oxidation of all kinds of energy materials and consequent nitrogen fixation, aided by phosphates.

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## FIXATION OF ATMOSPHERIC NITROGEN IN SAGAR SOIL

### Part II—With Energy-rich-materials added to Phosphate

By O. N. TRIPATHI and S. N. BANERJEE

*Department of Chemistry, University of Saugar*

(Received on 20th November, 1954)

Phosphorous is the most important element for the maintenance of animal, plant and marine life. Directly, it governs the most important physiological activities inside the cells; and indirectly, it has been observed that it controls the soil fertility to a great extent, through certain chemical and physico-chemical processes taking place in soil in presence of phosphates.<sup>1</sup> Thus, it has been observed that when phosphates are added to soil, nitrogen fixation occurs remarkably. Phosphate poor soils have been found to be poor in nitrogen. Dhar and co-workers<sup>2</sup> have found that in soils having a  $P_2O_5$  content of 0.075%, the efficiency of nitrogen fixation is much smaller than in soils having a  $P_2O_5$  content of 0.33–0.41%. Jules Joffe,<sup>3</sup> in 1902, claimed that the soils of Pyrenese fix nitrogen in presence of phosphate to the extent of 0.14%. In one of the reports of Sheila Dhar Institute of Soil Science, Allahabad, it is stated<sup>4</sup> "From a survey of soils of different countries it appears that nitrogen status of a soil is intimately connected with the phosphorous status all over the world. When the  $P_2O_5$  content is 1000 lbs per acre the nitrogen content cannot be greater than 0.1%, but when the  $P_2O_5$  content becomes 2000 lbs per acre or more the nitrogen content can go up to 0.2% or more. In other words, as  $P_2O_5$  content of soil increases, there is a marked increase of its nitrogen content. As a matter of fact, the increase of nitrogen content appears to be more steep with increasing amount of  $P_2O_5$  content in the soil." The Muck soils of New Jersey which contain 0.2% of  $P_2O_5$ , are shown to be rich in nitrogen to the extent of 2.7% in top 12" layer. The improvement of nitrogen status due to the phosphates present in soil has also been found in Rothamsted and Woburn Experimental Stations. By addition of farmyard manure at 14 tons per acre to Rothamsted soils the nitrogen status of the soil containing phosphates was greatly improved. But on adding artificial nitrogen no marked improvement in nitrogen status took place. On the other hand in more acidic soils of Woburn,<sup>6</sup> there was a very small increase of nitrogen even when 8 tons per acre farmyard manure was added to soil. This marked difference of nitrogen fixation in Rothamsted and Woburn Soils is supposed to be due to the presence of phosphates in the former which are useful for the purpose of increased fixation of nitrogen, unlike at Woburn where they are not available. E. Bear<sup>7</sup> has gone to the extent of saying thus about phosphates "phosphatic fertilizers within limits can be substituted for nitrogen fertilizers."

Thus, it has been finally established that the soils containing phosphates exhibit a remarkable behaviour of nitrogen fixation when they are exposed to sunlight. Dhar has definitely shown that when soils containing carbonaceous matter are allowed to undergo slow oxidation in air and light, a considerable

amount of atmospheric nitrogen is fixed in the soil. The amount of nitrogen thus added to soil is more in sunlight than in dark. The organic matter added or present in soil undergoes slow oxidation aided by sunlight and the presence of alkali, fixes nitrogen of the air and forms proteins in the process. Under ordinary conditions, the proteins formed undergo ammonification and nitrification which are also accelerated by light absorption to form nitrate :<sup>8</sup>

Between the stage of ammonia and nitrite, an unstable compound, ammonium nitrite, is formed which decomposes to give rise to free nitrogen gas.

Hence along with nitrogen fixation and formation of proteins due to oxidation of energy-rich-materials, ammonification and nitrification processes, which oppose the increase of proteins in soil, take place and thus the amount of protein remaining in the soil tends to decrease. Therefore, the apparent efficiency of nitrogen fixation seems to fall off when ammonification and nitrification processes take place. But the proteins which are present in soil humus are likely to be stabilized by the formation of nucleo-proteins and phospho-proteins side by side with the fixation of nitrogen in the system due to the presence of large amounts of phosphates in soils. This opposes the processes of oxidation leading to ammonia and nitrate formation, thus checking the nitrogen loss.

Russell<sup>9</sup> has also stated : "It is not known in what form the phytin or nucleic acids occur in organic matter though presumably most of the nucleic acid must be in nucleo-proteins nor in which humic fraction they are concentrated. They must be protected in some way from the soil enzymes as they are readily dephosphorylated if mixed with the soil *i. e.*, they have their phosphate groups split off as inorganic orthophosphate anions. This is also shown independently by the fact that these organic phosphates can only be extracted in good yield from the humic matter, if it is subject to fairly drastic pre-treatment."

In order to throw more light on the problem, we have undertaken here the study of the fixation of nitrogen in Sagar soil in presence of different organic materials, with and without phosphates.

#### EXPERIMENTAL

Soil was taken in glass jars and disodium-hydrogen phosphate was added to each of the jars in a quantity of 3 gms per lb of soil. Wheat straw and lignite were powdered well and were added separately in two pots in a quantity of 3 gms per lb of soil, sugar-candy was dissolved in water and added in a quantity of 3 gms per lb of soil in the third. Equal volumes of distilled water (50 c. c. per lb of soil) were added to all the jars and moisture content was kept practically constant. The sets were exposed to sunlight for about 8 hours everyday and analysed periodically.

We obtained the following results :—

Soil — B. No. 44 : Black, sticky, adheres to surface on wetting.

#### Results of Analysis.

Total carbon	1.1480%
Total nitrogen	0.1934%
Ammoniacal nitrogen	0.0015%

Nitrate nitrogen	0.0142%
Moisture content	1.472%
Loss on ignition	5.123%
Silica	74.467%
Sesquioxide	9.341%
Ferric oxide	3.981%
Sulphate	Traces.
CaO	2.3811
MgO	0.3462
K <sub>2</sub> O	1.2129
P <sub>2</sub> O <sub>5</sub>	0.3291

TABLE No. 1

3 gms of Lignite + 3 gms of Na<sub>2</sub>HPO<sub>4</sub> per lb of soil

Period of exposure	Total carbon %	Carbon oxidised %	Total nitrogen %	Change in nitrogen %
Original	1.9170	—	0.1958	—
7 days	1.9166	0.0013	0.1973	0.0015
15 „	1.8782	0.0397	0.1990	0.0032
30 „	1.6097	0.3082	0.2030	0.0072
60 „	1.2750	0.6429	0.2147	0.0189
80 „	1.0936	0.8243	0.2217	0.0259
100 „	1.0566	0.8613	0.2302	0.0344

Efficiency 39.8

TABLE No. 2

3 gms of Wheat straw + 3 gms of Na<sub>2</sub>HPO<sub>4</sub> per lb of soil

Original	1.7206	—	0.1950	—
7 days	1.7055	0.0151	0.2001	0.0051
15 „	1.5944	0.1262	0.2112	0.0162
30 „	1.4318	0.2888	0.2370	0.0420
60 „	0.9910	0.7296	0.2953	0.1003
80 „	0.7700	0.9506	0.2372	0.1322
100 „	0.5892	0.1314	0.3621	0.1671

Efficiency 145.9

TABLE No. 3

3 gms of Sugar = candy + 3 gms of  $\text{Na}_2\text{PHO}_4$  per lb of soil

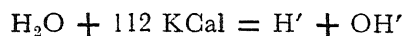
Original	1.7057	—	0.1957	—
7 days	1.6858	0.0189	0.1998	0.0041
15 "	8.6145	0.0912	0.2120	0.0163
30 "	1.4478	0.2579	0.2218	0.0261
60 "	1.1303	0.5749	0.2521	0.0565
80 "	0.9134	0.7923	0.2746	0.0789
100 "	0.7306	0.9751	0.2898	0.0941
Efficiency 96.6				

## DISCUSSION

Dhar and co-workers<sup>10</sup> have carried out extensive researches on nitrogen fixation for over 20 years by addition of different energy-rich materials to soils. They have definitely shown that considerable amount of nitrogen fixation takes place by allowing different energy-rich materials like sugar, starch, cellulose, fats, straw, leaves, lignite, etc. to undergo slow oxidation in air in presence of soil both in light and in dark and under sterile and unsterile conditions. They observed a marked fixation of nitrogen in light. G. V. Jacks,<sup>11</sup> in one of the reviews, also stresses upon Dhar's theory of photo-chemical nitrogen fixation. Bose<sup>12</sup> has studied the effect of artificial light upon photo-chemical nitrogen fixation in soil in presence of phosphates using straw and gur as energy producing materials. Their experimental results (with wheat-straw under unsterile conditions) show that soils fairly rich in calcium and phosphates fix considerable amount of atmospheric nitrogen, which reaches the order of 75-150 mgs of nitrogen per gm of carbon oxidised. They established that there is a marked light effect on nitrogen fixation. Other workers of Dhar's school found an efficiency of 10-25 mg in light with calcium and phosphate poor soils. Our experiments go to show that when phosphates along with other organic matter are added to soil and exposed to sunlight, the soil becomes sufficiently rich in nitrogen. When 3 gs of lignite, wheat straw and sugar-candy are added separately with 3 gm of di-sodium hydrogen phosphate per lb of soil and exposed to sunlight for 100 days 39.8, 145.9 and 96.6 mgs of nitrogen per gm of carbon oxidised are respectively fixed in the soil. Thus, straw, sugar candy and lignite stand in the order of efficiency as : straw > sugar-candy > lignite.

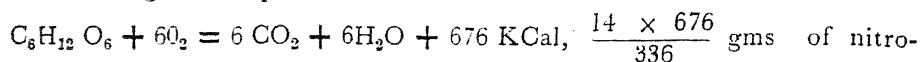
Dhar<sup>13</sup> has explained the phenomenon of photo-chemical nitrogen fixation as analogous to photo-synthesis in plants. He postulates that during the process of photo-chemical nitrogen fixation, decomposition of water molecules takes place by absorption of energy released in the oxidation of carbohydrates, cellulose, fats, lignite and wheat straw, etc.

Thus :



Now, for fixing 14 gs of nitrogen and forming ammonia by inter-action of molecular nitrogen and atomic hydrogen obtained by decomposition of water as

stated above, 336 KCal are needed. Hence by oxidation of a gram molecule of glucose, according to the equation :



gen can be fixed under ideal conditions. In other words, 0.39 gms of nitrogen should be fixed in such a reaction, per gm. of carbon oxidised under ideal conditions of the experiment. In our experiments with soil not poor in calcium and phosphate, a nitrogen fixation of 145.9 mgs with wheat straw, 96.6 mgs with sugar-candy and 39.8 mgs with lignite coal was obtained in light per gm of carbon oxidised. Therefore, the efficiency of this type of nitrogen fixation in soil

under unsterile conditions is  $= \frac{145.9 \times 100}{390} = 37.4\%$  with straw,  $\frac{96.6 \times 100}{390} =$

$24.7\%$  with sugar-candy and  $\frac{39.8 \times 100}{390} = 10.2\%$  with lignite coal.

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## DECOMPOSITION OF UREA AS SUCH AND IN PRESENCE OF GLUCOSE IN SAGAR SOIL

By O. N. TRIPATHI and S. N. BANERJEE

*Department of Chemistry, University of Sagar*

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It is well-known that nitrogen fixed or added as manure, does not remain for a long time in soil under ordinary circumstances. Though a large amount of nitrogen is constantly added to soil by fixation of atmospheric nitrogen under natural conditions, there is another process, active, side by side, which by certain chemical changes convert the nitrogenous matter of soil into free nitrogen which is lost to agriculture. This loss of added nitrogen from the soil has been found to be more with synthetic nitrogenous fertilizers than with natural manures. The loss of nitrogen in such a magnitude, is a great problem for the maintenance of soil fertility. Our agricultural practice should, therefore, be moulded in such a way as to utilise the maximum quantity of nitrogen added, if not the whole, by preventing the loss that is taking place.

Researches of Lipman and Blair,<sup>1</sup> Russell and Richards<sup>2</sup> and various other workers have definitely shown that nitrogen is lost in gaseous state when conditions are made favourable for oxidation. Snyder<sup>3</sup> reported a marked loss of nitrogen from Minnesota soils. Swanson<sup>4</sup> and Gainey<sup>5</sup> observed similar losses in Kansan soils. Russell<sup>6</sup> found a loss of nearly 70 % of the added nitrogen in wheat plots at Rothamsted receiving farmyard manure; and similar losses of nitrogen in Woburn plots have been reported by other authors. The loss was intensified by aeration. Further, Megitt<sup>7</sup> observed such losses occurring quite predominantly in humid soils of Assam. Annet, Aiyer and Kayastha<sup>8</sup> have shown a great loss of nitrogen to have taken place in black cotton soil of Nagpur, during heavy rains in the month of August. J. A. Voelcker<sup>9</sup> in "Improvement of Indian Agriculture" has reported "On looking into analysis of Indian soils . . . , I find that with the exception of the black cotton soils, Indian soils are generally deficient both in organic matter and nitrogen." The Royal Commission on Agriculture in India,<sup>10</sup> (1928) states "Of the principal plant food materials of which the soils of India are deficient, by far, the most important is nitrogen; and the manurial problem in India, is the main one of nitrogen-deficiency." The greater deficiency of nitrogen in Indian soils is said to be due to climatic conditions prevailing in the sub-continent. Jenny<sup>11</sup> has co-related the distribution of nitrogen and organic matter in soils to the climatic conditions and in another communication stated that for every 18°F fall in the mean annual temperature, the average nitrogen content of soil increases 2-3 times.<sup>12</sup>

Dhar and co-workers<sup>13-16</sup> have conducted a large number of experiments to study the process of ammonification through oxidative deamination and nitrification. Dhar, Tandon and Mukerjee<sup>17</sup> have observed losses of nitrogen by exposing solutions of ammonium salts to light. They have found bubbles of nitrogen gas escaping during such exposures. Dhar and Mukerjee<sup>18</sup> have shown that during

oxidation and nitrification of ammonium salts, there is always a loss of nitrogen gas. They have also shown that the process of nitrification and ammonification are partly photo-chemical and partly of surface reaction.

Dhar and Pant<sup>19</sup> have found considerable losses of nitrogen in soils from the decomposition of urea, and gelatin in complete absence of micro-organisms. Mukerjee<sup>20</sup> has reported that if the circumstances are favourable for oxidation, nitrogen loss takes place even under sterile conditions. Kapoor<sup>21</sup> has reported losses of nitrogen from solutions of urea during nitrification. The loss has been found to increase with temperature.

All the above observations go to show that nitrogen is being continuously lost from cultivated soils, of all types and in all kinds of practices, and from soils to which large amounts synthetic fertilizers are added. Soils, therefore, must possess some methods for gaining it, in an attempt to balance these losses.

The arable soils, if allowed to revert either to a natural forest or to pasture, gradually gains nitrogen and organic matter. A. D. Hall<sup>22</sup> found 0.037% increase of nitrogen after 22 years at Rothamsted by allowing the plots to revert to natural conditions.

Dhar and Mukerjee<sup>23</sup> have found that sterilized soils fix much nitrogen when exposed to light. Pant, Kapoor and Chatterjee, etc. have found that energy-rich materials like cellulose, green leaves, etc. fix nitrogen in soil. The decrease in losses of nitrogen in urea, hippuric acid, gelatin and ammonium salts have been observed, by Dhar's school, when carbonaceous materials are added to the soil. Subrahmanyam<sup>24</sup> has observed that addition of cellulosic materials is fairly effective in checking loss of nitrogen following additions of ammonium fertilizers to soils, specially under Indian climatic conditions. Dhar<sup>25</sup> and Mukerjee have shown beneficial results by adding molasses to fields to which ammonium sulphate is previously added.

In order to throw more light on the problem, we have undertaken here the study of the extent of decomposition of urea in Sagar soil in different concentrations; and the retardation in the loss of fertilizer by adding glucose to soil to which urea is previously added.

#### EXPERIMENTAL

Soil was taken in glass vessels; and different quantities of urea as such and with glucose were added to it. Three vessels containing 1, 3 and 5 gms urea along with 3 gms of glucose per lb of soil were regularly exposed to sunlight for about 10 hours every day. The sets were periodically analysed. The moisture was kept constant. The mean temperature during the exposure was 40°C.

We got the following results :—

#### ANALYSIS OF SOIL

	%
Total nitrogen in the soil	0.0712
Total carbon	0.6347
Ammoniacal nitrogen in the soil	0.0008
Nitrate nitrogen in the soil	0.0015
Nitrogen in urea	46.67



TABLE I

1 gm of Urea per lb of soil

Period of exposure	Total carbon per 100 gms of soil	Total nitrogen per 100 gms of soil	Ammoniacal nitrogen per 100 gms of soil	Nitrate nitrogen per 100 gms of soil	Per centage loss of nitrogen
Original	0.6347	0.1738	0.0008	0.0015	—
3 days	0.6344	0.1737	0.0437	0.0132	0.1
7 "	0.6340	0.1718	0.0425	0.0139	2.0
15 "	0.6305	0.1694	0.0422	0.0141	5.2
30 "	0.6266	0.1630	0.0413	0.0146	10.6
60 "	0.6180	0.1521	0.3098	0.0160	21.2
90 "	0.6101	0.1446	0.0352	0.0198	28.5
120 "	0.5665	0.1360	0.0312	0.0207	36.8
150 "	0.5461	0.1326	0.0219	0.0215	40.2
180 "	0.5173	0.1277	0.0213	0.0224	44.9
210 "	0.4987	0.1257	0.0205	0.0237	46.9
240 "	0.4689	0.1232	0.0203	0.0246	49.3

Mean decomposition of urea in unit time (1 month) 6.16%

TABLE II

3 gms of Urea per lb of soil

Period of exposure	Total carbon per 100 gms of soil	Total nitrogen per 100 gms of soil	Ammoniacal nitrogen per 100 gms of soil	Nitrate nitrogen per 100 gms of soil	Per centage loss of nitrogen
Original	0.6347	0.3777	0.0008	0.0015	—
3 days	0.6342	0.3755	0.0522	0.0165	0.4
7 "	0.6339	0.3688	0.0512	0.0172	2.9
15 "	0.6288	0.3547	0.0501	0.0187	7.5
30 "	0.6252	0.3431	0.0492	0.0200	11.3
60 "	0.6142	0.3029	0.0421	0.0206	24.4
90 "	0.5986	0.2833	0.0382	0.0216	30.8
120 "	0.5301	0.2554	0.0239	0.0258	39.9
150 "	0.5127	0.2416	0.0236	0.0287	44.4
180 "	0.4679	0.2266	0.0234	0.0325	59.3
210 "	0.4482	0.2177	0.0230	0.0342	52.2
240 "	0.4356	0.2100	0.0228	0.0355	54.7

Mean decomposition of urea in unit time (1 month) 6.84 %

TABLE III

5 gms of Urea per lb of soil

Period of exposure	Total carbon per 100 gms of soil	Total nitrogen per 100 gms of soil	Ammoniacal nitrogen per 100 gms of soil	Nitrate nitrogen per 100 gms of soil	Per centage loss of nitrogen
Original	0.6347	0.5786	0.0008	0.0015	—
3 days	0.6341	0.5751	0.0562	0.0182	0.7
7 "	0.6326	0.5598	0.0500	0.0187	3.7
15 "	0.6271	0.5375	0.0557	0.0199	8.1
30 "	0.6231	0.5142	0.0537	0.0215	12.7
60 "	0.6129	0.4507	0.0511	0.0245	25.2
90 "	0.5946	0.4213	0.0495	0.0283	31.2
120 "	0.5271	0.3660	0.0462	0.0287	41.9
150 "	0.5089	0.3800	0.0447	0.0290	47.3
180 "	0.4602	0.3051	0.0421	0.0331	53.9
210 "	0.4311	0.2919	0.0399	0.0352	56.5
240 "	0.4268	0.2762	0.0362	0.0361	59.6

Mean decomposition of urea in unit (1 month) 7.45 %

TABLE IV

Analysis of glucose. %			
Nitrogenous volatile matter	...	0.0020	
Chloride	...	0.0020	
Sulphate	...	0.0019	
Heavy metals (Pb)	...	0.0017	
Iron (Fe)	...	0.001	
Arsenic (As)	...	—	
Starch	...	—	
Moisture	...	0.4	
3 gms of Urea % 3 gms of glucose per lb of soil			
Period of exposure	Total carbon per 100 gms of soil	Total nitrogen per 100 gms of soil	Percentage loss of nitrogen
Original	0.9014	0.3753	—
3 days	0.8931	0.3748	0.18
7 "	0.8881	0.3717	1.10
15 "	0.8644	0.3637	3.80
30 "	0.8338	0.3558	6.40
60 "	0.7689	0.3454	9.85
90 "	0.7016	0.3352	13.20
120 "	0.6319	0.3172	19.10
150 "	0.5715	0.3028	23.50
180 "	0.5102	0.2944	26.60
210 "	0.4570	0.2843	29.90
240 "	0.4255	0.2756	32.80

#### Decomposition of urea in unit time (1 month) 4.1 %

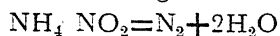
Our observations lead to the conclusion that when urea is added to soil and exposed to sunlight, the oxidation process sets in with a consequent decomposition of the fertilizer into free nitrogen gas through various stages of oxidation. The extent of this decomposition increases with the increasing quantities of the fertilizer. With 1 gm of the fertilizer per lb of soil, we have observed a loss of 49.3% of added urea in a period of eight months, whereas with 3 and 5 gms of the fertilizer 54.7% and 59.6 decomposition is observed. Thus the decomposition of urea in unit time (one month) comes to be 6.16%, 6.84% and 7.45% with 1, 3 and 5 gms of it respectively. Thus the mean percentage of decomposition of urea in Sagar soil under experiment is 6.82%. Dhar and co-workers<sup>26</sup> have reported a mean loss of 6.99% in light and 5.53% in dark in unit time.

When 3 gms of urea along with 3 gms of glucose were added to soil and exposed to sunlight as in case of urea for a period of eight months, we observed a considerable retardation in the loss of nitrogen from soil. Only 32.8% loss of nitrogen in the said period *i.e.*, 4.1% mean loss per month was observed with glucose.

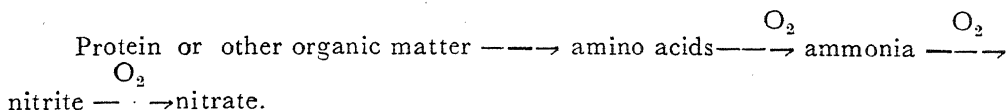
#### DISCUSSION

Dhar and co-workers<sup>27</sup>, for a number of years carried out extensive researches on slow oxidation of ammonium salts and other nitrogenous compounds in soil in presence of air. They observed that about 60% of the added nitrogen was lost due to oxidation by light and temperature. Russell<sup>28</sup> also reported a

recovery of only 43% of added nitrogen in the form of ammonium salts. Dhar and Narayanan<sup>29</sup> suggest that during the oxidation of the nitrogenous fertilizer in soil, the free ammonium ions which in their turn may further be oxidised to nitrate ions or may form a highly unstable intermediate compound, ammonium nitrite, decomposes to give rise to more of gaseous nitrogen as per equations :—



The whole mechanism of nitrogen transformation in soil has been generally accepted as taking place in the following manner<sup>30</sup> :—



Russell states<sup>31</sup> "In most arable soils, added ammonium ions are rapidly oxidised to nitrate; so no matter what form of nitrogen is given, nitrate is the only form present in appreciable concentration in the soil solution, for the plant to take up. Concentrated organic nitrogenous fertilizers such as urea, dried blood hoof and horns must decompose to ammonia first, which is usually oxidised to nitrate as rapidly as it is formed." He further states "Mineralisation of soil nitrogen is the name used for the purposes by which nitrogen in organic compounds become converted into inorganic ammonium and nitrate ions.

Hence there is always the possibility of ammonia and nitrate existing side by side which, therefore, together form ammonium nitrate, most of which decomposes to give free nitrogen gas. This loss is greater when larger amounts of fertilizers are used in the form of ammonium salts, for then the chance for the presence of larger quantities of ammonium nitrite in the stage of oxidation of ammonium salts is all the more prevailing.

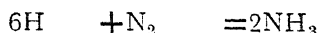
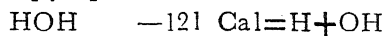
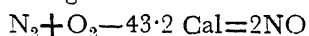
It is clear, therefore, that when ammonium salts or urea are added to soil, the first product formed is ammonia which in its turn is converted into its oxidised products like nitrate. Under these conditions at ordinary temperature, the whole of ammonia cannot be converted into oxidised products, quickly; and hence ammonium ions and occasionally free ammonia has to co-exist with nitrite ions or nitrous acid, nitrate ions or nitric acid, causing decomposition of urea with evolution of nitrogen gas.<sup>32</sup>

It has already been stated that addition of carbonaceous materials retards considerably the loss of nitrogen due to the decomposition of the added fertilizer. Our results (table IV) also show that there is a remarkable retardation in the loss of nitrogen of urea in presence of glucose in soil. The energy-rich-materials like celluloses, fats, carbohydrates etc. undergo oxidation.

Thus,<sup>33</sup>



This liberated energy is much more than needed for fixation of atmospheric nitrogen by either of the following mechanisms.



Thus, considerable nitrogen is fixed as ammonia which gets dissolved in the soil. The carbon of the energy-rich-material, therefore, when added to soil,

retards the velocity of oxidation of ammonium ions to nitrate, consequently decreasing the loss of nitrogen.

Further work on the retardation of the loss of nitrogen from nitrogenous fertilizers by addition of energy-rich-materials in Sagar soils is in progress.

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## ON THE PRECIPITATION OF IRON IN PEAT SOILS

By VILJO PUUSTJARVI, Dr. Agr.

*Department of Agricultural Chemistry, University of Helsinki, Helsinki, Finland*

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One-third of the land area of Finland about 10 millions hectares consists of bogs. Similarly, one-third of the cultivated area is made up of reclaimed bogs. The present, lively activity of clearing new arable land has the bogs for its primary objectives. It is thus only natural that the investigation of bogs and peat soils should constitute a very essential part of the research carried out in the field of soil science.

With a view to cultivation, the bogs are of considerably variable value. Various methods have therefore been developed, by means of which it is attempted to assess in advance the relative production capacity of the bogs. These methods are based, in the first place, on an investigation of the natural surface vegetation and of the peat of the bogs. The bogs have been classified in accordance with the surface vegetation into plant ecological types, the relative value in cultivation of which is known. The peats on the other hand are divided on the basis of their plant remains composition into macroscopically recognizable classes of fairly uniform chemical properties. In addition to this, conclusions with regard to the relative cultivation value of bogs can be drawn from other facts, *e.g.*, from the iron precipitates which are encountered rather frequently in bogs.

In connection with the podsolation phenomenon of soil, iron goes into solution from its surface layers and is not completely precipitated in the B horizon, but is partly carried into the ground water and with the latter also into the bogs. The major part of the bogs in Finland—particularly those in the northern part of our country, which is richer in bogs—are low-lying, wet swamps, so-called aapa bogs, where the occurrence of oxygen is limited to their highest layer of very small thickness, so that the iron remains in the reduced state in the peat layer and is only oxidized on the surface of the bog.

When ground water carries migrating iron from mineral soil to peat soil, the iron is entering an entirely different chemical environment. Ferrous iron can, in this environment, participate in numerous reactions. It can be precipitated as carbonate, phosphate or hydroxide. Because of its strong power to form complex compounds it has here an excellent opportunity to form such compounds. It can also form humates with humic acids and participate in cation exchange. Being in colloidal form, it can react with colloidal humus and silicic acid. It can also be oxidized and form several compounds as a trivalent ion.

When iron precipitates as carbonate it goes down as a grey mass as the ferrous carbonate does not form a colloidal solution. Usually, the iron precipitates which are to be found on the bottom of rimpis\* are brown gels which

\*Rimpi—a limited, water-logged bog area with mud bottom and practically without any moss vegetation. The size of the rimpi varies within wide limits, from about 10 centimetres to dozens of metres in diameter (Cajander, 1913). The sizes of the rimpis in the study have varied from a diameter of about 10 to 40 cm.

contain iron both in ferrous and ferric forms. The gels in question can be formed either from ferrous carbonate oxidized to ferric hydroxide or, by the precipitation of ferrous iron as ferrous hydroxide followed by its partial oxidation to the ferric state, or by the ferrous iron first being oxidized to ferric form and thereafter precipitating as ferric hydroxide.

The precipitates of iron are found chiefly on the bottom of the rimpis which act as precipitation troughs. In the hummock banks around the rimpis ferrous carbonate is not to be found at a corresponding depth (Kivinen, 1936).

On the surfaces of the rimpis waters, one will usually find "oily" films. Mostly these films are very thin, but sometimes they are of such thickness that it is quite easy to take a sample of them on one's hand. In this case the substance will remain on the surface of the hand as particles of brown colour or with a gold or silvery sheen.

Frequently there are iron precipitates on the bottom of these "oily" rimpis. In general, the formations in question are found especially in the eutrophic mires. The pH of the water from the "oily" rimpis varied in the 81 investigated cases in such a way that 5 samples were in the pH range 6, 20—6, 29, 56 in the pH range 6, 30—6, 39, 17 in the pH range 6, 40—6, 49 and 3 in the pH range 6, 50—6, 59. On the basis of the determinations performed it seems quite probable that the formation of the "oily" surface films implies a fairly high pH. The pH values of the waters of 49 rimpis in which no iron precipitates occurred varied from 4.05 to 5.45.

Since the substance in question occurred, as a rule only in rimpis where an iron precipitate was found on the bottom, it seemed possible that the "oily" substance might be a definite stage of the iron precipitation. When this question was studied it was found that the substance was dissolved by hydrochloric acid and that it included both ferrous and ferric iron.

Although the water of the "oily" rimpis proved to have quite a high pH, this is no indication that the pH of the peat on the bottom of the rimpis would be similarly high. The pH of the peat of the "oily" rimpis has not been classified separately, but these determinations are included in the pH determinations of the bottom peat which contains the iron precipitates. The samples were taken from the bottom of the rimpis from a depth of about 0-10 cm. The greater number of the rimpis studied were very small rimpis of the kind encountered in most of the types of bog. In Table I are shown the results found with the abovementioned study material.

TABLE I

The pH values of iron-containing peat soils of rimpis					
pH	Number of samples	pH	Number of samples	pH	Number of samples
5.40-5.49	17	6.30-6.39	15	7.10-7.19	5
5.50-5.59	132	6.40-6.49	12	7.20-7.29	6
5.60-5.69	141	6.50-6.59	48	7.30-7.39	10
5.70-5.79	68	6.60-6.69	16	7.40-7.49	4
5.80-5.89	23	6.70-6.79	23	7.50-7.59	2
5.90-5.99	27	6.80-6.89	21	7.60-7.69	...
6.00-6.09	16	6.90-6.99	18	7.70-7.79	1
6.10-6.19	32	7.00-7.09	9	7.80-7.89	4
6.20-6.29	37				

It should be noted that the samples of the study material have been taken from bogs that are in the natural state in which the surface of the bogs has not become dry.

The pH of the peat of rimpi containing iron precipitates varied over quite a wide range, but it has not been below 5.5. The samples below this limit have mostly been borderline cases in which the rimpi has become dry or the occurrence of iron precipitation has been questionable. In the evident cases, the pH has not been lower than 5.46.

On the basis of my observations during four summers there exists a quite distinct relation between the iron precipitates and the quality of the bogs. Bogs where definite iron precipitates have been seen on the surface, have belonged at least to the quality class\* 5 or higher.

Iron can be supposed to precipitate in a rimpi either as hydroxide or as carbonate. In order to find out which type of precipitation finds more favourable conditions in rimpis, the pH,  $E_h$  and the content of  $\text{CO}_2$ ,  $\text{HCO}_3'$  and  $\text{Fe}^{++}$  in some rimpis were determined.

Table 2 shows the results of the analysis of a rimpi. Three of the determinations have been performed upon "oily" rimpis, but the fourth analysed specimen was "oil free."

When the oxidation-reduction potentials are examined, a distinct difference between the various types of rimpis can be seen. In the "oil free" rimpi, the potential is so low that the ionized iron is practically completely in the ferrous state. The potassium thiocyanate reagent in no case showed the presence of ionized ferric iron.

TABLE 2  
Equilibrium values of rimpi waters

Type and No. of rimpi	$E_h$ mv	pH	$\text{H}_2\text{CO}_3$ $\times 10^{-4}$	$\text{HCO}_3'$ $\times 10^{-3}$	$\text{Fe}^{++}$		Ks $\times 10^{-19}$	$\text{Fe}^{++}$ $\times 10^{-4}\dagger$
					ekv/l $\times 10^{-3}$	mol/l $\times 10^{-1}$		
"Oily" rimpi 1	226	6.31	3.8	1.50	1.46	7.3	3.80	12.0
" " " 2	236	6.42	4.2	1.00	0.90	4.5	3.09	6.6
" " " 3	244	6.39	5.3	1.20	1.10	5.5	3.31	6.0
"Oil-free" " 4	372	5.88	1.0	0.10	0.38	1.9	0.11	331.2

The amounts of bicarbonate and ferrous iron are almost equal. This fact easily leads to the hypothesis that all of the soluble ferrous iron occurs in the form of bicarbonate, but since iron also has occurred in other forms at lower pH, in this case even at pH 5.88 (Table 2, rimpi 4), this hypothesis cannot be considered probable.

\* Virgin productivity—(German "Bonitat") refers to soil-use capability; classes from 1 to 10, the classes from 5 to 10 being tillable.

† Calculated necessary  $\text{Fe}^{++}$  concentration for precipitation as carbonate.

By supposing that the "oil" is one of the precipitation stages of iron and that, in this case, the iron has been precipitated as a ferrous hydroxide, the following equation can be applied :

$$(\text{Fe}^{+2}) \times (\text{OH}')^2 = K_s$$

The calculated values for  $K_s$  are also presented in Table No. 2. It is seen that these values are approximately equal to those obtained from the titration curve for the titration performed in air (Puustjärvi, 1952). On account of this it seems to be possible that the iron in the investigated rimpis has been able to precipitate at least partly as ferrous hydroxide.

In the case in question, in addition to hydroxide the iron can also be assumed to be precipitated as a carbonate. To clarify the equilibrium  $\text{Fe}^{+2} - \text{CO}_3''$ , the  $\text{Fe}^{+2}$  concentrations which would cause the iron to precipitate as carbonate in the rimpis presented in Table No. 2 are calculated by using the equation :

$$\text{pFe} + \log (\text{H}_2\text{CO}_3) + 2 \text{pH} - 6.28$$

Table No. 2 also gives these calculated values. At a comparison of the determined values and the calculated values of the  $\text{Fe}^{+2}$  concentration in Table No. 2 it appears that the real values of the ferrous ion in the "oily" rimpis are in part about equal, but generally smaller than those consistent with the balance between  $\text{Fe}^{+2} - \text{CO}_3''$ . This indicates that iron in some cases may have been partly precipitated as carbonate. Since the precipitation as hydroxide was more probable, it is likely that iron has been precipitated as ferrous hydroxide or as basic ferrous carbonate.

A distinct difference is noted between the "oily" and "oil-free" rimpis, when the conditions prevailing in them are compared. The difference in their content of carbonic acid as well as those of iron are rather large. The data in Table No. 2 show that iron in the "oil-free" rimpis was not capable of precipitation as hydroxide and carbonate. The comparison of the "oily" and "oil-free" rimpis on the basis of this instance indicates that the "oily" surface films constitute one stage of the iron precipitation.

On the basis of the studies presented above, the conditions in "oily" rimpis seem to have been such that iron has had theoretical possibilities to be precipitated, principally as hydroxide but partly also as carbonate. Consequently it may be most likely that iron has been precipitated primarily as a hydroxide, though partly also as a basic carbonate in the same way as happens in the titration of ferrous chloride with sodium carbonate where the hydroxide and carbonate are precipitated in the same pH-zone (Puustjärvi, 1952).

A number of hypotheses have been put forward concerning the formation of siderite (van Bemmelen 1899, Tacke 1923).

The conditions in which the precipitation of ferrous carbonate is possible are : Low acidity and high carbon dioxide pressure. Obviously the variation in the pressure of carbon dioxide in the mire water is relatively smaller than that of pH. In the following the hydrogen ion concentration of the siderite profile is taken into consideration.

Table No. 3 gives the oxidation-reduction potentials and the pH values of two siderite profiles.



TABLE No. 3

pH values of two siderite profiles

Profile No. 1				Profile No. 2			
Depth m	Kind of peat	E <sub>h</sub> mv	pH	Depth m	Kind of peat	E <sub>h</sub> mv	pH
0 —0.1	LCp*	244	7.10	0 —0.3	LCp*	154	7.24
0.1 —0.2	„	192	7.53	0.3 —0.6	„	144	8.50
0.2 —0.25	Sider	164	8.11	0.6 —0.8	„	38	7.40
0.3 —0.4	„	146	>8.50	0.8 —1.0	Sider	—98	>8.50
0.4 —0.5	Sider	134	>8.50	1.0 —1.2	LCp*	10	8.38
0.5 —0.6	LCp*	164	6.44	1.2 —1.5	„	40	7.00
0.6 —0.7	„	164	7.06	1.5 —1.7	„	62	6.49
0.7 —0.8	„	154	8.10	1.7 —1.8	Sider	60	8.04
0.8 —0.85	Sider	120	>8.50	1.8 —1.9	„	50	6.49
0.85—1.0	Sider	96	>8.50	1.9 —2.1	„	30	7.50
				2.1 —2.15	„	—42	>8.50

The oxidation-reduction potentials of the peats are low, especially those of the siderite samples. Although, theoretically, the potential of pure ferrous salt should be —, the potential of the purest ferrous salt solution in practice is scarcely lower than 400 mv. Therefore, the low oxidation-reduction potentials of the siderite profiles cannot depend on the ferric-ferro system. Perhaps some sulfuric compounds have, in this case fixed the potential of the system.

Iron is also to be found in peat soils as iron phosphate or vivianite. This, in a reduced state, is a light brown or yellowish mass. Vivianite mostly occurs together with siderite. It is very difficult, if not impossible, to separate these two compounds. When vivianite has been in contact with air and oxidized, it first becomes blue, then greyish blue and finally brown. In practice, vivianite is identified on the basis of its blue colour. It is, therefore, usual to consider as vivianite any bluish masses found in peat soils. In this study all bluish masses are considered to contain vivianite.

Table No. 4 gives the pH values of a vivianite profile. Some vivianite and siderite were present throughout the whole peat profile.

Some pH measurements were also made in other vivianite profiles. In all cases the reactions of the vivianite samples have been neutral or slightly alkaline.

It has already been mentioned that the vivianite is known by its blue colour which appears when the sample is not in contact with air. When ferrous iron is precipitated with alkali, this precipitate also becomes partly blue. It seems obvious that in both cases the appearance of the same colour depends on the same factors.

\* Wood-sedge post,

TABLE No. 4

The  $E_h$  and pH values of a vivianite profile.

Depth m	$E_h$ mv	pH fresh	pH dry
0 —0.2	248	7.33	4.28
0.2—0.4	250	7.27	4.21
0.4—0.6	254	6.98	4.19
0.6—0.8	250	6.86	4.01
0.8—1.0	300	6.52	4.10
1.0—1.2	330	6.42	4.16
1.4—1.6	340	6.40	3.97
1.6—1.8	330	6.42	3.24
1.8—2.0	320	6.56	2.65
2.0—2.2	250	7.20	
2.2—2.4	295	7.81	
2.4—2.6	290	7.10	
2.6—2.8	285	7.05	
2.8—2.9	290	7.13	

The experiments of Wilder, Bancroft and Cunningham, (1930) have shown that only iron compounds which contain both ferrous and ferric iron are blue. The ratio of the ferrous iron to the ferric iron varies within relatively wide limits dependent upon the acid radical and other substances present in the compounds.

The most generally accepted explanation of the blue colour often found in clays is that such a colour is produced by finely distributed organic matter, the basis for this belief apparently being the general occurrence of organic matter in such blue clays (ref. Wilder, Bancroft and Cunningham, 1930). Since, however the research done by the above-mentioned workers has shown that the blue colour appears in numerous ferro-ferric compounds which contain no organic matter, there is no doubt that it is the iron rather than the organic matter which is responsible for the blue colour, the latter type of material probably serving merely to prevent further oxidation of the iron.

MacCarthy, (1926) has shown that the mixture of ferrous and ferric solutions, when no reaction takes place between them, never seems to give rise to a blue colour and that the addition of a proper precipitant to such a mixed solution will invariably deposit a blue precipitate, provided no excess of ferric iron is present. It appears certain that these blue iron compounds are not mere mixtures of ferric and ferrous salts, but true ferro-ferric compounds.

Because the blue colour is caused by the ferro-ferric ratio it depends only upon the total amount of ferrous and ferric iron and has no connection with their forms of combination. Thus a ferro-ferric phosphate might well have the same ratio as a mixture of ferrous carbonate and ferric hydroxide. If such is the case, the blue colour cannot be considered to be any certain indication of vivianite.

Professor Mauno J. Kotilainen has kindly placed his unpublished material on vivianite and siderite analyses at my disposal. According to these analyses the  $P_2O_5$  content of vivianite and siderite samples were as follows :—

Vivianite	0.24	0.45	1.40	2.40	3.54	3.83	4.70	9.92%
Siderite	0.13	0.21	0.36	0.57	1.12	2.13%		

For the sake of comparison it may be mentioned, moreover, that the  $P_2O_5$  content of peats varies on an average from 0.08 to 0.21%, according to Kivinen, (1938,1954).

The figures presented show that vivianite samples richest in phosphate contain considerably more  $P_2O_5$  than do the siderite samples. However, it is also seen that the  $P_2O_5$  contents of vivianite samples poorer in phosphorus belong to the the same class as those of the siderite samples, and that the  $P_2O_5$  content of the vivianite and siderite samples which have the lowest  $P_2O_5$  content does not differ much from the phosphate content of peats.

The purpose of this study was to elucidate to what degree the iron precipitates can be used as indicator of the fertility of bogs. To elucidate the phenomenon in question field observations have been made during four summers. It has been established that iron precipitates are to be found only in such bogs where the virgin productivity is 5 or more. With numerous pH measurements it has been found that iron precipitates only occur in bogs in which the pH of the peat is about 5.5 or higher. The pH and the quality of the peat are associated with each other in such a manner that bogs with pH higher than 5.5 can be assumed to belong to the quality class 5 or to a still higher class.

My laboratory experiments (Puustjärvi, 1952) and the field studies show that iron can be precipitated only at a high pH. The "oily" surface films, which are generally found in rimpi occurred, in the investigated cases, only within the pH interval of 6.2—6.6. The field observations have also shown that such films occurred only in bogs of the quality 6 or higher.

It has been established both by the theoretical calculations and by the field observations that siderite and vivianite can occur only in peat of slightly acid or alkaline reaction. The quality of such bogs can be assumed to be 7 or even higher.

Since the field observations, the chemical determinations and the theoretical consideration of the phenomena indicate that iron precipitates can occur only in such bogs whose virgin productivity is 5 or more, the formations in question can be regarded, with great certainty, as indications of the tillability of such bogs.

#### SUMMARY

The purpose of the study has been to elucidate the factors which give rise to the precipitation of iron as inorganic compounds in bogs and also to determine whether these occurrence can be used as indications of the virgin productivity of bogs. The main results are as follows :—

1. The "oily" surface films in rimpi waters appear to be formed when the ionized iron is in the precipitation phase. In the cases studied the precipitation occurred in the pH-zone 6.2—6.6.

2. The pH of the peat which contains an iron precipitate, is at least 5.4 but usually over pH 5.5.
3. Iron is precipitated in rimpis as hydroxide. The precipitation as carbonate is also possible, obviously as a basic compound.
4. Siderite is formed in peat soils only in a neutral or slightly basic reaction.
5. The blue colour of ferrous phosphate or vivianite has been assumed to be caused by a certain ferro-ferric ratio. In addition to phosphate, some other compounds, for example carbonate, can stabilize the ferro-ferric ratio which produces the blue colour of this compound.
6. The iron precipitates can be regarded as indications of the tillability of the bogs. The thick "oily" surface films of rimpi waters seem to be signs of a virgin productivity class 7 or higher.

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# MODELLSUBSTANZEN VON HUMINSÄUREN

VON W. FLAIG\*

*Institut für Biochemie des Bodens der Forschungsanstalt für Landwirtschaft  
Braunschweig-Völkenrode*

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Bei der Konstitutionsaufklärung hochmolekularer Naturstoffe sind schon seit jeher entsprechende Modellsubstanzen herangezogen worden. So versucht man auch der chemischen Konstitution von Huminsäuren näherzukommen durch Modellsubstanzen, die durch alkalische Oxydation von Polyphenolen hergestellt werden.

Die natürlichen Huminsäuren besitzen Redoxcharakter und bei ihrer Oxydation erhält man Stoffe, die auf chinoide bzw. phenolische Bausteine schließen lassen. In der Alkalischemelze erhält man Protocatechusäure und Brenzcatechine<sup>1</sup>, bei der Oxydation mit Brom Tetrabromchinon<sup>2</sup> und bei der mit Chlordioxyd Maleinsäureanhydrid.<sup>3</sup> Beim Erhitzen mit Nitrobenzol in alkalischer Lösung wird, wenn auch in geringen Mengen, die Bildung von Vanillin<sup>4</sup> beobachtet.

Nicht nur durch Oxydation, sondern auch durch Hydrierung werden phenolische Bestandteile erhalten. Mit Nickelkatalysatoren konnten Phenolbasen,<sup>5</sup> mit Molybdänsulfid als Katalysator<sup>6</sup> Propyl-Cyclohexanolderivate isoliert werden. Die Stickstoffentwicklung bei der Umsetzung mit Phenylhydrazincarbam<sup>7</sup> lässt auf die Gegenwart von chinoiden Gruppen schließen.

Als eines der möglichen Ausgangsprodukte für die natürliche Bildung von Huminsäuren im Boden wird das Lignin angesehen. Die Bausteine des Lignins sind zum grössten Teil Phenoläther, deren Methoxylgruppen durch die Mikroorganismen des Bodens aufgespalten werden.<sup>8</sup> Hierdurch besteht die Möglichkeit der Bildung von Polyphenolen, die auf einem ähnlichen Wege weiter kondensieren können, wie es bei der Bildung der Synthesuminsäuren der Fall ist.

Über die Autoxydation von Hydrochinon in alkalischem Medium wurden schon mehrere Arbeiten veröffentlicht. Insbesondere wurde diese zuletzt von A. Weissberger und Mitarbeitern<sup>9</sup> näher beschrieben. Diese Autoren stellten fest, dass als Zwischenprodukt Oxy-p-benzochinon entsteht.

Der Weg zu den in weiteren Reaktionen entstandenen Synthesuminsäuren ist noch völlig unbekannt. In jüngster Zeit haben auch E. Welte und Mitarbeiter<sup>10</sup> Untersuchungen über die Autoxydation von Hydrochinon gemacht, bei

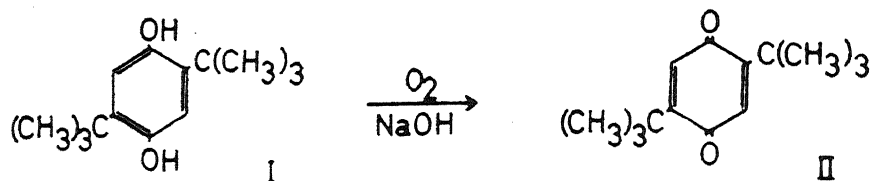
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\*Anschrift: Braunschweig, Bundesallee, 50.

der sie als Endprodukt Oxalsäure und Essigsäure beschrieben haben, ohne Zwischenprodukte angeben zu können. Ebenfalls befassten sich H. G. H. Erdtman und M. Granath<sup>11</sup> mit der Einwirkung von Alkali auf Benzochinon.

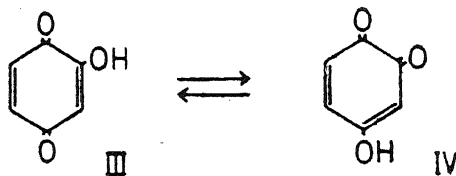
Im Gegensatz zu seiner geringen Stabilität in schwach alkalischer Lösung gegenüber Sauerstoff ist Hydrochinon in stark alkalischen Lösungen verhältnismässig beständig und kann erst durch Wasserstoffsuperoxyd zum 2, 5-Dioxychinon aufoxydiert werden. Während das Mono-Oxy-p-benzochinon eine sehr instabile Verbindung ist, ist das 2, 5-Dioxy-p-chinon verhältnismässig beständig. Die Substitution in 2, 5-Stellung bewirkt daher eine Stabilisierung des Moleküls.

Wir untersuchten aus diesem Grunde die Oxydation von 2, 5-disubstituierten Chinonon und fanden bei der alkalischen Autoxydation von 2, 5-Dimethylhydrochinon und Thymohydrochinon zwar noch braunschwarze, den Huminsäuren sehr ähnliche Verbindungen, die jedoch im Gegensatz zu den aus dem unsubstituierten Hydrochinon gewonnenen Synthesehuminsäuren und im Gegensatz zu den natürlichen Huminsäuren in Äther löslich waren.



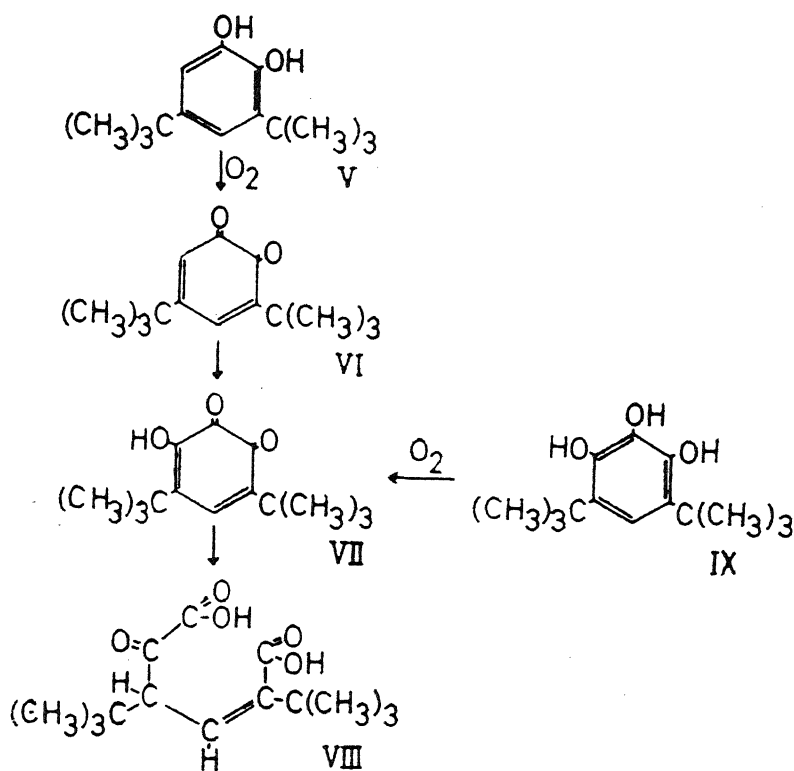
Bei der Substitution des Hydrochinons mit Tertiärbutylgruppen in 2, 5-Stellung (I) blieb Reaktion unter den gewählten Bedingungen beim Chinon (II) stehen, das aus der Lösung ausfiel. Es scheint demnach so zu sein, dass bei derartigen, substituierten Chinonen kaum und in manchen Fällen fast keine dehydrierende Polymerisation stattfinden kann, wie sie bei der Bildung von Huminsäuremodellen als Polymere des Oxychinons von verschiedenen Autoren<sup>12, 13</sup> angenommen wurden.

Es war weiterhin die Frage zu klären, auf welchem Wege die Bildung der Synthesehuminsäuren aus dem Hydrochinon erfolgen könne. Die-o-Chinone werden in den meisten Fällen durch Oxydationsmittel rascher aufgespalten als die p-Chinone<sup>14</sup>.



Es war nun die Frage, ob vom Oxy-p-benzochinon (III) eine tautomere Form als 4-Oxy-o-benzochinon (IV) besteht, die während des Reaktionsverlaufes leicht aufgespalten werden kann. Wir überprüften<sup>15</sup> diesen Gedanken und fanden, dass das für p-Chinone gefundene Absorptionsmaximum bei ca. 240m/ $\mu$  und das für o-Chinone bei ca. 380m/ $\mu$  gefundene Maximum jeweils charakteristisch ist. Oxychinone, wie Oxythymochinon und andere, besaßen 2 Maxima,

deren Lage jeweils der o- und der p-Chinonkonfiguration entsprach, und die uns für eine o-p-Tautomerie charakteristisch erscheinen.



Wir gingen<sup>15</sup> daher dazu über, auch das 3, 5-Di-tert. butylbrenzcatechin (V) einer alkalischen Oxydation zu unterwerfen. Als Endprodukt wurde die 2, 4-Di-tert. butyl-oxalocrotonsäure (VIII) erhalten. Die Reaktion verläuft über das 3, 5-Di-tert. butyl-o-benzochinon (VI) und über das 2-Oxy-3, 5-di-tert. butyl-o-benzochinon (VII), die beide für sich dargestellt werden konnten und bei der Spaltung jeweils die Oxalocrotonsäure ergaben.

Auch das 4, 6-Di-tert. butyl-pyrogallol (IX) ergab bei der Autoxydation 2, 4-Di-tert. butyl-oxalocrotonsäure.

Bei der Bildung der Synthesehuminsäuren findet demnach sehr wahrscheinlich eine Ringaufspaltung statt. Nach dem augenblicklichen Stand der Kenntnisse ist noch nicht mit Sicherheit auszusagen, ob die Huminsäuren nur aus ringförmigen Verbindungen aufgebaut sind oder ob die Kondensation der Spaltprodukte zu deren Bildung führt. Sehr wahrscheinlich werden die Huminsäuren sowohl aus ringförmigen als auch aus aliphatischen Bausteinen aufgebaut sein. Sinngemäß lassen sich diese Reaktionen auch auf die Spaltstücke des Lignins übertragen.

Weiterhin wäre noch die Frage zu diskutieren, ob nicht Sauerstoffbrücken zwischen den einzelnen Ringen, auftreten, wie sie z. B. in jüngster Zeit bei der Bildung von Dehydrodigallussäure<sup>16</sup> und Valoneasäure<sup>17</sup> gefunden wurden.

Bei Synthesehuminsäuren, die in alkalischer Lösung hergestellt werden, werden Sauerstoffbrücken nicht vorhanden sein können, da Äther dieser Art in alkalischer Lösung durch Sauerstoff aufgespalten werden.

Derartige Untersuchungen mit Modells-substanzen zeigen auf, dass die Konstitutionsaufklärung natürlicher Huminsäuren mit vielen Schwierigkeiten verknüpft ist.

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# EXTENSION DES ECHANGES ISOTOPIQUES D'IONS $\text{PO}_4$ DANS LE SOIL, PAR ALTERNANCES DE DESSICCATION ET D'HUMECTATION, ET PAR EBULLITION

Par G. BARBIER ET MELLE E. TYSZKIEWICZ

*Station Centrale d'Agronomie, Versailles*

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## I FAITS EXPERIMENTAUX

Cette communication repose essentiellement sur le fait expérimental suivant :

Un petit poids de terre (quelques g) est agité en présence d'une solution phosphatée neutre (100 c.c.), contenant  $^{32}\text{PO}_4\text{H}_3$  de concentration en  $\text{P}_2\text{O}_5$  variable, mais toujours très diluée pour éviter la précipitation de phosphates calciques. Après un certain temps d'agitation (12h), la solution est filtrée, et la terre est desséchée pendant 4 h à  $40^\circ\text{C}$ . On agite ensuite à nouveau la terre avec le filtrat, et ainsi de suite. Dans d'autres essais parallèles, la suspension est agitée pendant le même temps, mais sans dessiccations. Dans une 3<sup>ème</sup> série, la suspension est soumise à 10 minutes d'ébullition, puis agitée 24 h.

Dans tous les sols examinés, les alternances de dessiccation et d'humectation, comme l'ébullition, ont provoqué simultanément une diminution de la radioactivité en solution et une diminution de la radioactivité spécifique de  $\text{P}_2\text{O}_5$  en solution. La diminution de la radioactivité en solution implique une rétention supplémentaire par le solide d'ions  $\text{PO}_4$  initialement présents dans la solution. La diminution de la radioactivité spécifique de  $\text{P}_2\text{O}_5$  en solution implique un mouvement inverse, c'est-à-dire que des ions  $\text{PO}_4$  préexistants dans le solide et n'ayant pas encore participé aux équilibres cinétiques, se sont mélangés avec ceux de la solution, la "dilution isotopique" des ions  $^{33}\text{PO}_4$  parmi les ions  $^{32}\text{PO}_4$  et des  $^{31}\text{PO}_4$  parmi les  $^{32}\text{PO}_4$ , intéresse une plus grande masse de  $^{31}\text{PO}_4$  préexistants.

La masse totale des ions  $\text{PO}_4$  du sol (en phase solide ou en solution) participant de façon permanente ou ayant participé temporairement aux équilibres cinétiques, que nous appellerons  $\text{P}_2\text{O}_5$  isotopiquement échangeable, peut-être calculée par la formule :

$$\frac{\text{radioactivité totale}}{\text{P}_2\text{O}_5 \text{ isotopiquement échangeable préexistant} + \text{P}_2\text{O}_5 \text{ ajouté} *} = \frac{\text{radioactivité en solution}}{\text{P}_2\text{O}_5 \text{ en solution}}$$

Cette formule signifie qu'à l'équilibre les ions  $^{32}\text{PO}_4$  et  $^{31}\text{PO}_4$  sont distribués rigoureusement au hasard entre les 2 phases dans la même proportion de  $^{32}\text{PO}_4$  et  $^{31}\text{PO}_4$  dans chaque élément de volume).

\* Ce terme est nul si l'on ajoute  $^{32}\text{PO}_4\text{H}_3$  sans entraîneur.

A vrai dire, ce mode d'expression des résultats n'est pas absolument exact, car l'équilibre est rarement atteint au bout de quelques jours. Appliquer la formule en cours d'évolution du système revient à supposer que les  $^{32}\text{PO}_4$  ajoutés ont formé à l'instant considéré un mélange homogène avec  $\text{P}_2\text{O}_5$  isotopiquement échangeable défini par la formule. En réalité, tant que l'équilibre n'est pas atteint, le rapport  $^{32}\text{PO}_4/^{31}\text{PO}_4$  dans chaque élément de volume doit diminuer d'une manière continue dans l'espace dans la direction de la diffusion de  $^{32}\text{PO}_4$ , c'est-à-dire vers l'intérieur des particules en l'espèce. La masse des  $\text{PO}_4$  du sol atteints par la diffusion des  $^{32}\text{PO}_4$  peut donc être plus grande que ne l'indique la formule.

1.2 g de terre + 100 c.c d'eau +  $^{32}\text{PO}_4\text{H}_3$  sans entraîneur ;

3 alternances de dessiccation et d'humectation, ou 10 mn d'ébullition

Sols du Champ d'expériences de Versailles	Parcelle à super- phosphate	Parcelle à scories	Témoin sans en- grais phosphaté
mg $\text{P}_2\text{O}_5$ par Kg	sans dessic- cation ni ébull	sans dessic- cation ni ébull	sans dessicca- tion ni ébul- lition.
$\text{P}_2\text{O}_5$ isotopique- ment échangea- ble	350	520 571	267 432 538 88
$\text{P}_2\text{O}_5$ en solution	48,5	52 104	89 58 71 ...
$\text{P}_2\text{O}_5$ soluble aux acides dilu- és		236	481 59
$\text{P}_2\text{O}_5$ soluble aux alcalis di- lués		471	197 117
Somme des deux précédents		707	678 176

2.5 g de terre + 100 c.c. sol. Phosphatée contenant 7,15 mg  $\text{P}_2\text{O}_5$  +  $^{32}\text{PO}_4\text{H}$  ajouté avant ébullition ; puis 24 h d'agitation.

mg  $\text{P}_2\text{O}_5$  per Kg

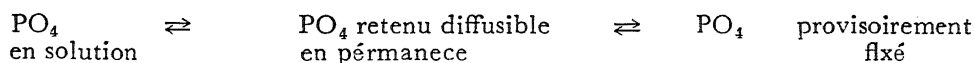
		$\text{P}_2\text{O}_5$ retenu	$\text{P}_2\text{O}_4$ isotopique- ment échangeable préexistant
Limon de Versailles pH final = 6,0	{ sans ébull. avec ébull.	251 { I 495 } II 465 } moy : 580	126 203 } moy : 188 173 }
Terre de Toulouse pH final = 5,8	{ sans ébull. avec ébull.	231 { I 637 } II 583 } moy : 610	276 722 } moy : 731 741 }

Les alternances de dessiccation et d'humectation ont augmenté  $\text{P}_2\text{O}_5$  isotopi-  
quement échangeable de 50 et 60 % (I er tableau). Par ébullition, l'augmentation  
est respectivement 70 %, 100 %, 49 %, 170 %.

Par ailleurs, le premier tableau montre qu'une fraction importante de  $P_2O_5$  des engrais été réellement fixée dans ces sols, c'est-à-dire ne participe pas actuellement aux équilibres.

## II INTERPRETATION DES RESULTATS

D'après ces faits, l'ensemble du phénomène de rétention et de libération des ions  $PO_4$  pourrait être représenté par le schéma suivant, analogue à celui qui a été proposé pour  $P_2O_5$  ou  $K_2O$  :



La double flèche de gauche correspond à des échanges permanents entre la solution libre et la phase solide, plus rapides pour les surfaces externes que pour des surfaces internes, celles-ci restant toutefois en communication permanente avec la solution libre. La double flèche de droite correspond à des échanges n'ayant lieu, (ou n'atteignant une vitesse notable) qu'en certaines circonstances, notamment au cours des alternances de dessiccation et d'humectation, ou pendant l'ébullition. Les ions  $PO_4$  fixés seraient retenus dans des prisons dont les portes de communication avec l'extérieur ne s'ouvriraient que dans les dites circonstances. Si l'on a augmenté récemment l'activité des ions  $PO_4$  à l'extérieur (par un apport d'engrais) l'ouverture des portes provoquerait une diffusion d'ions  $PO_4$  dans les prisons, et ces ions resteraient fixés après la refermeture des portes. Inversement, si des cultures ont prélevé récemment des ions  $PO_4$  sur l'extérieur, l'excès d'activité à l'intérieur provoquerait une diffusion vers l'extérieur lors de l'ouverture des portes. Effectivement, si  $^{32}PO_4H_3$  est ajouté après les alternances de dessiccation, et d'humectation ou après l'ébullition celles-ci ont beaucoup moins d'effet. Par ailleurs, les alternances de dessiccation et d'humectation, comme l'ébullition, en même temps qu'elles ont provoqué une extension temporaire des échanges isotopiques à de nouvelles masses d'ions  $PO_4$  du sol, ont tantôt augmenté tantôt diminué la masse de  $P_2O_5$  en solution. Inpossibilité de libération d'ions  $PO_4$  par dessiccation—humectation est connue (Lebediantzeff, Schloesing fils et D. Leroux)\*. Mais les résultats précédents montrent que les mêmes circonstances peuvent aussi bien provoquer une fixation qu'une libération de  $P_2O_5$ . Le cas de la terre de Toulouse est particulièrement significatif à cet égard. L'ébullition a augmenté la quantité de  $P_2O_5$  prise par le sol la solution de 231 à 610 mg par Kg, alors que la quantité des ions  $PO_4$  préexistant dans le sol ayant pris part aux échanges a augmenté de 276 à 731 mg  $P_2O_5$  par Kg. Il serait difficilement concevable que l'augmentation de la masse  $P_2O_5$  prenant part aux équilibres puisse être accompagnée d'une diminution de  $P_2O_5$  en solution, si l'on n'admettait pas que des surfaces supplémentaires, portant déjà des ions  $PO_4$  échangeables, mais susceptibles d'en retenir davantage, sont entrées en jeu. Voici d'ailleurs un autre exemple où des dessiccation alternant avec des humectations ont favorisé la rétention des ions  $PO_4$  :

25 g de sous sol de limon + 1 litre de solution neutre de phosphate calcique contenant 15 mg  $P_2O_5$ .

durée d'agitation (jours)	...	1	3	6	8	10	13	15
mg $P_2O_5$ { sans dessiccations	...	6,4	4,15	4,36	3,26	3,08	2,66	2,4
restés en { avec 1 dessic. entre chaque								
solution { mesure	...	6	3,1	2,3	2,1	1,8	1,7	1,7

\*d'après A. DEMON. Dynamique du sol, 2<sup>ème</sup> Edition, p. 382.

Après 6 jours, il reste environ 2 fois moins de  $P_2O_5$  en solution en présence de la terre qui a subi 3 dessications, ce qui peut correspondre grosso modo à une augmentation du simple au double des surfaces ayant contribué à la rétention.

### III CONCLUSION

Les faits observés confirment que pour augmenter le masse des ions  $PO_4$  diffusibles du sol (ou échangeables, ceux que concerne le double fléch de gauche) il est nécessaire de dépenser plus de  $P_2O_5$  que si le pouvoir de fixation du sol n'existait pas. Mais inversement, dans un sol qui s'apeuvrit, une fraction des ion  $PO_4$  fixés antérieurement est susceptible de repasser à l'état diffusible. Fixation et libération apparaissent come deux aspects d'un seul et même phénomène : une extension temporaire des échanges d'ions à des surfaces qui ne participent pas normalement aux equilibres cinétiques. En difrinitive, il s'agit toujours d'équilibres, et nous n'avons pas de preuve que si l'on a fourni à un sol une certaine quantité de  $P_2O_5$  sous forme soluble, et que l'on en ait ensuite retiré par des cultures la même quantité, l'activité des ions  $PO_4$  puisse demeurer définitivement en dessous de son niveau initial, ce qui sereit le cas si la fixation partielle de  $P_2O_5$  incorporé était définitive (ceci sans tenir compte de la formation éventuelle d'apatites ou autres associations irréversibles. Par ailleurs tout anrichissement du sol en  $P_2O_5$  par apport de phosphates susceptibles de s'y dissoudre, doit augmenter de façon permanente l'activisé de ses ions  $PO_4$ , plus ou moins selon le pouvoir de rétention ou defixation du sol ce qui n'éteit d'ailleurs guère douteux d'après les observations chimiques antérieures.

SYSTEMATIC INVESTIGATION OF SOIL ORGANIC MATTER WITH  
PARTICULAR EMPHASIS ON THE SOIL HYDROLYSATES  
OF AGRA SOIL

**Part III—Alkali hydrolysate of Agra Soil and preliminary  
investigations on its organic components**

By B. R. NAGAR, NEWTON RAM and ABANI K. BHATTACHARYA

*Department of Chemistry, Agra College, Agra*

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In our previous publication<sup>9</sup> and in continuation of the communication<sup>11</sup> of this series on the acid hydrolysates of Agra soil experiments were carried out on the alkali hydrolysates also to elucidate further the chemical aspects of humus by analysing the insoluble fractions. In the acid hydrolysates<sup>11</sup> of the unmanured soil of the Botanical Garden of Agra College, we were able to detect in some of the extracted fractions the presence of glycollic acid, galactose, cellobiose and very small amounts of gummy resinous matter (unidentified). In this paper it is to communicate our findings on the nature of organic components obtained by analysing the various humic fractions extracted by treating the Agra soil with semi-normal caustic soda solution refluxed on sand bath for six hours. The percentage of carbon in the soil has also been quantitatively studied.

EXPERIMENTAL

It is now quite a well-known fact that a great variety of complexes have been reported to exist in the humic fractions of the soil obtained by adopting the method of alkali hydrolysis with suitable modifications by different authors<sup>1, 2, 13—15</sup>. In our investigations we have followed the method of Schreiner and Shorey approved by the Bureau of Soils in Washington.

The alkali hydrolysate in our experiments was prepared by refluxing the mixture of soil and alkali at the boiling temperature. The scheme of extractions of the various humic fractions obtained by different solvent actions and by evaporation is mentioned in Chart I that follows. The percentage of organic carbon was determined by Black and Walkley's method<sup>13</sup> in each of the extracts—soluble and insoluble—obtained from the humic fractions. Nitrogen was estimated by Kjeldahl's<sup>8</sup> method in all the soluble and insoluble fractions. The presence of nitrogen, sulphur and halogens and the acid or alkaline nature of the fractions were also detected. The fractions obtained in different solvents were subjected to group tests and necessary inferences were drawn, wherever possible.

# CHART I

*Showing the various humic fractions (soluble and insoluble) by alkali hydrolysis of Agra soil*

7 kilograms of soil refluxed with 7 litres of N/2 NaOH on sand bath

for six hours and filtered

Insoluble I  
Humus coal or humin  
(Sprengel, Berzelius and Page)

Filtrate I  
Treated with N/2  $H_2SO_4$   
refrigerated 24 hours and filtered.

Insoluble II  
Humic acid (Shmuk) %C 27.145,  
% N 5.748  
N, S and Cl present, acidic.  
Refluxed with 95% alcohol  
for 6 hours and filtered.

Filtrate II  
Brown mass on evaporation.  
Fulvic Acid (Oden & Tyurin)  
%C 2.82, %N 2.943  
N and S present, halogen  
absent. Further properties  
in the table ahead.

Insoluble III  
%C 33.97, %N 8.84, N,  
S and Cl present, acidic.  
Soxhletised with benzene  
for 20 hours.

Filtrate III  
Hematomelanic acid.  
(Hoppe-Seyler, Euler and Oden)  
Brown substance on evaporation.  
%C 31.3, %N 12.36  
N and S present, halogen absent,  
acidic. Extracted with petroleum  
ether and filtered.

Wax-like residue associated  
with very small amount of  
brown substance obtained  
from the extract. Melting  
point of one part of the  
residue 60°C, another part  
130°C a residue remained  
which, did not melt even  
after 190°C.  
Further work in progress.

Insoluble IV  
N and S present halogen  
absent, acidic.

Filtrate IV  
A very small amount  
of substance obtained  
on evaporation. N, S  
and halogen absent,  
purely organic, no  
residue on burning on  
Nickel foil and acidic.

## CHART II

*Showing the percentage of Carbon in the various fractions with reference to the total Carbon in the Agra Soil*

Soil, containing 0.5082 % of organic Carbon, was refluxed  
with N/2 NaOH for six hours and filtered

Insoluble I  
Represents 86.75 % of  
the total carbon.

Filtrate I  
Alkaline solution  
contains 13.43 % of  
the total Carbon

acidified with H<sub>2</sub>SO<sub>4</sub>  
and filtered.

Insoluble II  
Precipitate contains  
6.59 % of total carbon  
Refluxed with absolute  
alcohol and filtered.

Filtrate II  
Acid filtrate contains  
6.84 % of total carbon

Insoluble III  
contains 4.02 % of  
total carbon.

Filtrate III  
Alcoholic solution contains  
2.50 % of total carbon.

### TABLE

#### TREATMENTS

Filtrate II	Concentrated aqueous soln. extracted with ether	Soxhletised with benzene for 20 hours and eva- porated	Extracted with a mixture of chloroform and ether, distillate evaporated to dryness	Steam distilla- tion 15% HCl and the distil- late examined
Fulvic acid				
On evapora- tion on water bath a brown mass was ob- tained which was used for all treatments <sup>4</sup>	The etherial extract on evaporation gave a very small quanti- ty of purely organic yellow mass (N, S and halogens absent) Being too small in quantity de- tailed exami- nation was not possible.	A very small quantity of yel- low mass. N, S and halogens absent. Fehlings solution reduced. Molisch test positive. Purely organic subst- ance, the quan- tity being too small, detailed examination was impracticable.	Yellow mass in the centre and crystals around it. N, S and halogens absent acidic in nature purely organic, decolourises acidified KMnO <sub>4</sub> CaCl <sub>2</sub> test for oxalic acid res- ponded. Evi- dence for the persence of the shape of crystals round the yellow mass obtained.	1. Odour resem- bles solution of Furfuraldehyde. 2. Reduces Feh- ling solution. 3. Aniline ace- tate paper turned red. 4. Molish's test positive. Presence of fur- furaldehyde confirmed in the distillate. Hence pentosan, pen- toses or uronic acids are present in the fulvic acid fraction.

## DISCUSSION

In discussing the results of our experiments on the alkali hydrolysates, a few striking points need be mentioned from the observations already communicated on the work of acid hydrolysates of Agra soil in the part I and part II of this series.

In part I of this series<sup>3</sup>, we reported the fractions obtained by the hydrolysis of the soil with N/2 HCl. In the scheme given therein a dark brown sticky mass (insoluble matter I) which gradually separated during the concentration of the acid hydrolysate contained N, S and Cl. It was further reported in part II of this series that the aforesaid insoluble matter I treated separately with solvents-acetic acid, acetone-alcohol, alcohol-ether, left an insoluble fraction which was a brown residue also giving the tests for N, S and Cl in all cases. In the scheme of alkali hydrolysis (Chart I) it will be seen that the humic acid fraction (insoluble III) also contains N, S and Cl. It appears, therefore, on the evidence of the elementary analysis that there is some analogy of the humic acid fraction (insoluble III) obtained from the alkali hydrolysate with the insoluble fraction I of the acid hydrolysate.

In Chart I, the various humic fractions obtained by the treatments mentioned therein have been given. The estimations of carbon and nitrogen in the relevant fractions show that the percentages of carbon and nitrogen are the highest in the last extractions, that is, insoluble III and filtrate III fractions. It is rather interesting that while S and Cl are both present in the insolubles II and III, only S is present in the soluble filtrates II and III. It suggests therefore that the insoluble fractions shown in the chart might be either halogenated compounds or mixed halogenated compounds with inorganic chlorides as distinguished from the filtrates which contained N and S but did not give any tests for halogens. Very extensive work is yet necessary to resolve the complexities in such fractions and ultimately confirm the nature of these components.

The next item of interest regarding the alkali hydrolysates of Agra soil will be found in chart II where the percentages of carbon in various fractions with reference to the total Carbon in the soil have been calculated and shown. It will be evident from this chart that only 13.43 % of the total organic carbon comes in the alkaline hydrolysate and the insoluble unhydrolysed fraction (humic or humus coal) contains 86.57 % of the total carbon in the soil. This is very significant to indicate that soil organic matter is composed of two types of humic matter, one of which is chemically active, while the other is more resistant. Reference may be made here of similar observations given by the sponsors of the theory of Nauer humus and Dauer humus which is supported by the chemical analysis of the soil organic matter even under drastic treatment of hydrolysis. It is further to be noted that the humic acid fraction in Agra soil contains only 6.59% of total carbon while fulvic acid contains 6.84 % of total carbon. It appears from the above that the fractions of humic and fulvic acids may be almost equal in the Agra soil. Further interest arises over the insoluble and soluble fractions of humic acid when it is refluxed with absolute alcohol. The insoluble fraction of humic acid consists of 4.02 % and the alcohol soluble fraction consists of 2.5% of total organic carbon of the soil. From this observation it naturally follows that the so-called humic acid is a mixture of soluble and insoluble components in absolute alcohol.

The characteristics of the fulvic acid fraction present, by far, the most interesting feature of our investigations in this paper. It contains N and S, and

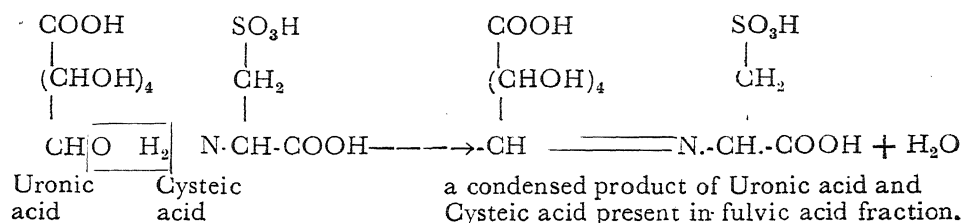


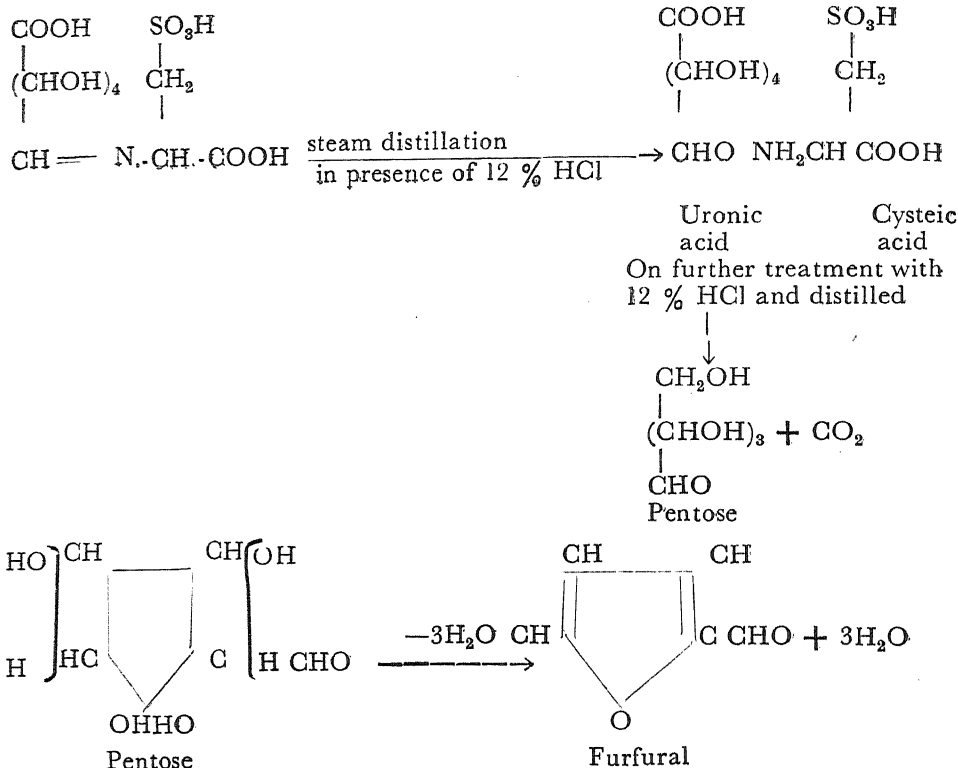
the brown mass on treatment with different solvents gives very small quantities of pure organic substances (*vide* the table). By chemical test the extract in chloroform and ether showed the presence of oxalic acid which was also isolated by Shorey from the acidified filtrate of the alkaline extract of the soil and also by treating the soil with dilute HCl. It may also be mentioned that we detected glycollic acid in the HCl-hydrolysates of Agra soil. Shorey<sup>18</sup> suggested that this acid is present in the form of calcium oxalate and the abundance of oxalic acid is introduced into the soil by the various plant residues and is also produced by variety of fungi and bacteria which ultimately decompose it to carbon dioxide and fulvic acid.

By steam distillation of the brown mass (fulvic acid) with 12% HCl the presence of furfural was detected in the steam distillate. This observation suggests that pentoses or pentosans or uronic acids are the components of fulvic acid fraction. Similar observation have been made by previous authors<sup>5,7,9,14,17,18</sup> regarding the presence of pentosans in the humus of peat, and of forest and mineral soil.

Tyurin<sup>18</sup> suggests that the fulvic acid groups of soil organic matter may be looked upon as products of condensation of carbohydrates and uronic acids with the intermediate products of the decomposition of protein and he stated further that the possible causes and mechanism of such a reaction was still an open question.

Since we observed that fulvic fraction of Agra soil contained N and S and also gave furfural, it bids fair that the mechanism of condensation should constitute a combination of uronic acid with a suitable compound containing nitrogen and sulphur. Very little, however, is known about sulphur content of organic matter or the form in which it occurs. The reference in the literature shown that Evans, Rost<sup>6</sup> and Madonov<sup>10</sup> worked on the quantitative aspect of nitrogen, sulphur and carbon sulphur ratios of the Minnesota soils. It has been suggested that some of the sulphur presumably occurs as the amino acids, cystine and cysteine, which are present in the proteins derived from the plant residues. They do not separate from the humic material as they are very resistant to hydrolysis. Bremner<sup>1</sup> detected as many as 20 amino acids in acid hydrolysates by partition chromatography. He observed that amino acid components of different soils were remarkably similar and he could detect the oxidation products of cystine (cysteic acid) and Menthionin sulphone on paper chromatograms of acid hydrolysates. In the light of the above references we may propose the following mechanism, which may, *a priori*, explain the decomposition of the fulvic acid complex into furfural by steam distillation.





Extensive work is in progress in these laboratories to resolve the complexities of humic fractions of Agra soil by the study of hydrolysates.

#### SUMMARY

Preliminary investigations on the alkali hydrolysates of Agra Soil have been made. The elementary detections of the various humic fractions have been reported. All the humic fractions were found to be of acidic nature. It has been suggested that the humic acid fractions of the acid and alkali hydrolysates are more or less analogous. The percentages of carbon and nitrogen in the alcohol insoluble III and the alcohol soluble hematomelanic acid filtrate III were found to be higher than the percentages of the humic acid and the so-called fulvic acid.

The decomposable or chemically active carbon in the soil is only 13.43 % of the total carbon. The alkaline hydrolysate on being acidified precipitates humic acid which contains 6.59 % of total carbon and the filtrate contains only 6.84 % of total carbon suggesting that humic acid and fulvic fractions may be present in equivalent amounts in Agra soil. In the chloroform and ether extract of fulvic acid fractions, oxalic acid was detected and the steam distillate of fulvic acid in 12 % HCl, furfural was confirmed by standard tests. A possible explanation of the formation of furfural from uronic acid has been given and a mechanism of the reactions yielding furfural has been suggested on the basis of a condensed product of uronic acid and oxidation product of an amino acid containing sulphur such as cysteic acid.

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# THE PHOTO-CHEMICAL DEGRADATION OF $\alpha$ —, AND $\beta$ —ALANINE AND ITS IMPORTANCE IN SOIL CHEMICAL PROCESSES\*

By C. S. VAIDYANATHAN, G. D. KALYANKAR and K.V. GIRI

*Department of Biochemistry, Indian Institute of Science, Bangalore-3*

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## INTRODUCTION

The formation of ammonia from amino acids as a result of irradiation by ultra-violet light and X-rays has been reported by several workers.

Lieben and Urban (1931) found that only the  $\alpha$ -amino-group is attacked by ultra-violet light. They also obtained evidence to show that there might be secondary reactions of amino acids, especially in the case of histidine and tryptophan, in addition to the splitting of ammonia.

In his classical work, Neuberg (1910) exposed solutions of amino acids, peptides and proteins to sunlight in the presence of oxygen and metal salts. The products were found to be aldehydes, ammonia and carbon dioxide.

Weizmann *et al* (1936) observed that when aqueous solutions of glycine, alanine and aspartic acid were exposed to ultra-violet light, hydrolysis occurs giving the corresponding hydroxyacids and ammonia.

The action of sunlight on aqueous solutions of amino acids, however, has not been studied in detail.

Gopala Rao and Dhar (1934), while investigating the photolysis of amino acids found that some amino acids are oxidized with the liberation of ammonia on exposure to sunlight in presence of various sensitizers. In most cases the amount of ammonia-N formed was found to correspond quantitatively to the decrease in amino-N.

In previous publications from this laboratory (Giri, Kalyankar and Vaidyanathan, 1953, 1954), it was reported that the photolysis of amino acids in sunlight in presence of  $\text{TiO}_2$  was not a simple case of oxidative deamination as was presumed by previous workers but represented a complicated process giving rise to a number of intermediate products many of which could not be identified with certainty.

In this paper are given the details of our quantitative studies on the photolysis of  $\alpha$ —, and  $\beta$ —alanine by sunlight in the presence of photosensitizers.

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\*The work reported in this paper formed part of the thesis submitted by one of us (C. S. V) for the Ph. D. degree of the Madras University.

These two amino acids were selected for detailed study because the reactions of these amino acids were comparatively simple. Both these amino acids gave only glycine as an intermediate, the end-products being ammonia and probably nitrite. A critical discussion of the importance of these reactions in soil chemical processes is also included.

#### EXPERIMENTAL

The amino acids used in the present investigation were obtained from Nutritional Biochemicals Corporation and were used without further purification.

The photosensitizers employed were of A. R. quality (either B. D. H. or Merck).

0.5 c.c. of 0.1 M. solution of each amino acid was diluted to 5 c.c. with double-distilled water and exposed to sunlight in presence of 10 mg of the photosensitizer in Pyrex Conical Flasks. Similar flasks were also kept in the dark to serve as blanks. Controls were also run in sunlight in the absence of catalysts. In all cases it was found that the amino acids were quite stable when exposed to sunlight without the addition of photosensitizers.

The degradation products were identified by the circular paper chromatographic technique of Giri and Rao (1952, *a* and *b*). The identity of the products was confirmed by running separate circular paper chromatograms with different solvents (*e.g.*, phenol saturated with water and pyridine-water 80 : 20) and also by the use of 2-dimensional paper chromatographic technique.

The amino acids were estimated after separation by circular paper chromatography according to the method of Giri, Radhakrishnan and Vaidyanathan (1953).

In some cases ammonia-N was estimated by the microdiffusion method of Conway (1940).

#### RESULTS AND DISCUSSION

$\alpha$ -alanine was comparatively resistant to photo-chemical degradation and was slowly decomposed to glycine, while  $\beta$ -alanine was easily attacked the only product detected being glycine.

##### *Effect of various sensitizers and the time of reaction on the photolysis of $\alpha$ —, and $\beta$ -alanine.*

Experiments were carried out with various sensitizers and exposing the solutions for different intervals of time.

5 c.c. of 0.01M solutions of the amino acids were exposed to sunlight in pyrex conical flasks under sterile conditions. Each day the exposure lasted for 7 hours from 8-30 A. M. to 3-30 P. M. At convenient intervals, 20 $\mu$ l aliquots were taken out from each flask for chromatographic analysis.

From the results given in Tables I *a* and *b*, it was found that titanium dioxide was the most efficient of the photocatalysts used and that there was a gradation in the velocity of the reaction with increase in the concentration of TiO<sub>2</sub>. The mode of degradation was similar with the different photocatalysts.

Generally it was found that the amount of amino acids disappeared could not be accounted for by the amount of glycine and ammonia produced. This indicates that the ammonia is further oxidized probably up to the nitrite stage.

TABLE I a

Photolysis of  $\alpha$ -alanine in sunlight in presence of various sensitizers

Sensitizer	Time of exposure in hours	$\alpha$ -Alanine in mg	Glycine formed in mg	NH <sub>4</sub> —N in mg
—	7	4.2	—	—
TiO <sub>2</sub> (10 mg)	7	3.75	Traces	—
Al <sub>2</sub> O <sub>3</sub> (10 mg)	7	4.0	—	—
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	7	4.0	—	—
—	21	4.1	—	—
TiO <sub>2</sub> (10 mg)	21	3.2	0.15	—
Al <sub>2</sub> O <sub>3</sub> (10 mg)	21	4.0	—	—
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	21	4.0	—	—
HgO (10 mg)	21	2.5	—	—
—	35	4.1	—	—
TiO <sub>2</sub> (10 mg)	35	2.9	0.2	—
TiO <sub>2</sub> (10 mg)	49	2.2	0.15	—
TiO <sub>2</sub> (10 mg)	59	2.0	0.25	—
TiO <sub>2</sub> (10 mg)	73	1.4	0.17	—
TiO <sub>2</sub> (10 mg)	87	1.1	0.10	—
TiO <sub>2</sub> (10 mg)	101	0.7	0.13	—
TiO <sub>2</sub> (10 mg)	115	0.30	—	0.34

TABLE 1b

Photolysis of  $\beta$ -alanine by sunlight in presence of various sensitizers

Sensitizer	Time of exposure in hrs	$\beta$ -alanine in mg	Glycine formed in mg	NH <sub>4</sub> —N in mg	Remarks
TiO <sub>2</sub> (10 mg)	7	4.2	—	—	In dark
—	7	4.0	—	—	In light
TiO <sub>2</sub> (5 mg)	7	4.0	0.17	—	“
„ (10 mg)	7	3.9	0.22	—	“
„ (20 mg)	7	3.8	0.30	—	“
HgO (10 mg)	7	4.1	—	—	“
Al <sub>2</sub> O <sub>3</sub> (10 mg)	7	4.1	—	—	“
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	7	4.0	—	—	“
—	14	4.0	—	—	“
TiO <sub>2</sub> (5 mg)	14	3.5	0.28	—	“
TiO <sub>2</sub> (10 mg)	14	3.5	0.35	—	“
„ (20 mg)	14	3.0	0.33	—	“
HgO (10 mg)	14	3.7	Traces	—	“
Al <sub>2</sub> O <sub>3</sub> (10 mg)	14	3.9	—	—	“
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	14	3.8	—	—	“
—	21	4.0	—	—	“

Sensitizer	Time of exposure in hrs	$\beta$ -alanine in mg	Glycine formed in mg	NH <sub>4</sub> -N in mg	Remarks
TiO <sub>2</sub> (5 mg)	21	3.2	0.52	—	In light
" (10 mg)	21	2.8	0.60	—	"
" (20 mg)	21	2.6	0.60	—	"
HgO (10 mg)	21	3.7	Traces	—	"
Al <sub>2</sub> O <sub>3</sub> (10 mg)	21	3.9	—	—	"
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	21	3.8	—	—	"
—	28	4.0	—	—	"
TiO <sub>2</sub> (5 mg)	28	3.0	0.70	—	"
" (10 mg)	28	2.7	0.70	—	"
" (20 mg)	28	2.1	0.60	—	"
HgO (10 mg)	28	3.5	0.16	0.05	"
Al <sub>2</sub> O <sub>3</sub> (10 mg)	28	3.6	—	Nil	"
Fe <sub>2</sub> O <sub>3</sub> (10 mg)	28	3.6	—	Nil	"
TiO <sub>2</sub> (5 mg)	42	2.2	0.85	—	"
" (10 mg)	42	1.8	1.00	—	"
" (20 mg)	42	1.25	0.85	—	"
" (20 mg)	49	0.6	0.3	0.34	"
" (10 mg)	56	0.9	0.6	—	"
" (10 mg)	70	0.4	0.5	—	"
" (10 mg)	77	0.3	0.7	0.41	"

*Effect of concentration of amino acid*

The effect of concentration of the amino acid on the rate of photolysis was determined maintaining the other conditions the same. It was found that the amount of glycine produced increased with increase in the initial concentration of  $\beta$ -alanine (Table II) the volume of the reaction mixture in all cases was 5 c. c.

TABLE II  
Effect of concentration of  $\beta$ -alanine on the photolysis in the presence of TiO<sub>2</sub> (10 mg)

Initial concn. of $\beta$ -alanine mM/litre	Time of exposure in hours	Glycine formed in mg	NH <sub>4</sub> -N in mg
4	7	0.2	—
10	7	0.22	—
20	7	0.28	—
40	7	0.32	—
4	14	0.26	—
10	14	0.35	—
20	14	0.48	—
40	14	0.59	—
4	21	0.40	—
10	21	0.60	—
20	21	0.80	—
40	21	0.87	—
4	28	0.33	0.17
10	28	0.77	—
20	28	0.94	—
40	28	0.99	—
20	49	0.94	0.41
40	49	1.24	0.46

### Effect of pH

The effect of pH and the nature of the buffer used on the photolysis of these amino acids was next studied. The reaction mixtures consisted of 0.5 c. c. of 0.1M amino acid solution and 4.5 c. c. of appropriate buffer and 10 mg of  $\text{TiO}_2$ .

The yield of glycine from  $\beta$ -alanine was maximum at pH 5.0 (acetate buffer). At pH 7.7, the rate of photolysis of  $\beta$ -alanine was less in presence of borax buffer than when either phosphate or veronal buffer was used (Table No. IIIb), This was observed in the case of  $\alpha$ -alanine also (Table IIIa).

TABLE IIIa

Effect of pH on the photolysis of  $\alpha$ -alanine in presence  
of  $\text{TiO}_2$  (10 mg)

$\alpha$ -alanine in mg	Time in hours	pH and buffer	Glycine formed in mg	$\text{NH}_4\text{—N}$ in mg
3.8	24	3.7 (Acetate)	0.1	—
3.5	24	5.0 (Acetate)	0.1	—
2.8	24	7.7 (Veronal)	—	—
3.1	24	7.7 (Borax)	—	—
1.9	24	9.3 (Borax)	—	—
3.5	38	3.7 (Acetate)	0.21	—
3.1	38	5.0 (Acetate)	0.23	—
2.2	38	7.7 (Veronal)	Faint band	—
3.0	38	7.7 (Borax)	0.17	—
1.5	38	9.3 (Borax)	0.10	—
3.1	52	3.7 (Acetate)	0.30	—
2.8	52	5.0 (Acetate)	0.32	0.32
1.4	52	7.7 (Veronal)	—	0.48
2.5	52	7.7 (Borax)	0.20	—
1.0	52	9.3 (Borax)	—	—
2.6	66	3.7 (Acetate)	0.35	—
2.0	80	3.7 (Acetate)	0.42	0.52

TABLE IIIb

Effect of pH on the Photolysis of  $\beta$ -alanine .  $\text{TiO}_2$  added in  
each case 10 mg

Time in hrs.	pH and buffer	$\beta$ -alanine in mg	Glycine formed in mg	$\text{NH}_4\text{—N}$ in mg
7	3.7 (Acetate)	3.9	0.2	—
7	5.0 (Acetate)	3.1	0.25	—
7	7.7 (M/15 phosphate)	3.9	0.17	—
7	7.7 (Veronal)	3.7	0.17	—
7	7.7 (Borax)	3.9	0.13	—
14	3.7 (Acetate)	3.2	0.35	—
14	5.0 (Acetate)	2.6	0.51	—
14	7.7 (Phosphate)	3.1	0.35	—



Time in hrs.	pH and buffer	$\beta$ -alanine	Glycine formed in mg	NH <sub>4</sub> -N in mg
14	7.7 (Veronal)	3.4	0.47	—
14	7.7 (Borax)	3.6	0.28	—
28	3.7 (Acetate)	2.4	0.70	—
28	5.0 (Acetate)	1.8	0.92	—
28	7.7 (Phosphate)	2.2	0.50	—
28	7.7 (Veronal)	2.4	0.65	—
28	7.7 (Borax)	2.6	0.58	—
42	3.7 (Acetate)	1.6	1.06	—
42	5.0 (Acetate)	1.45	1.45	—
42	7.7 (Phosphate)	1.3	0.70	—
42	7.7 (Veronal)	1.2	0.86	—
42	7.7 (Borax)	1.7	0.81	—
49	3.7 (Acetate)	0.9	0.6	0.63
49	5.0 (Acetate)	0.7	0.8	0.63
49	7.7 (Veronal)	0.52	0.32	—
49	7.7 (Borax)	1.3	0.45	—

*The effect of Metallic Ions*

The importance of trace elements in the economy of the soil and in plant growth is well-known. Therefore a study was made of the effect of some divalent metallic ions like manganese and molybdenum on the rate of photolysis of these amino acids. The concentration of the metallic ions was always  $50 \mu\text{g M}^{++}/\text{c. c.}$  of reaction mixture where  $\text{M}^{++}$  represents the divalent metallic ion. Cobalt and manganese ions were found to decrease the rate of degradation of both  $\alpha$ - and  $\beta$ -alanine to a great extent (Table IV *a* and *b*).

TABLE IV *a*

Effect of divalent metallic ions on the photolysis of  
 $\alpha$ -alanine

*TiO<sub>2</sub> added in each case 10 gm*

$\alpha$ -alanine in mg	Metal salt soln. added	Time of exposure in hrs	Glycine formed in mg	NH <sub>4</sub> -N in mg
3.5	—	7	Traces	—
3.8	MnSO <sub>4</sub>	7	Traces	—
3.5	Ammonium molybdate	7	Traces	—
3.5	ZnSO <sub>4</sub>	7	Traces	—
3.2	—	21	0.15	—
3.7	MnSO <sub>4</sub>	21	Traces	—
3.2	Molybdate	21	0.23	—
3.0	ZnSO <sub>4</sub>	21	0.20	—
2.9	—	35	0.20	—
3.7	MnSO <sub>4</sub>	25	Traces	—

<i>a</i> -alanine in mg	Metal salt soln. added	Time of exposure in hrs.	Glycine formed in mg	NH <sub>4</sub> -N in mg
3.0	Molybdate	35	0.20	—
2.5	ZnSO <sub>4</sub>	35	0.23	—
1.9	—	59	0.25	—
3.4	MnSO <sub>4</sub>	59	Traces	—
1.8	Molybdate	59	0.20	—
1.4	ZnSO <sub>4</sub>	59	0.10	—
1.0	—	87	0.10	—
3.1	MnSO <sub>4</sub>	87	Traces	—
1.2	Molybdate	87	0.2	—
0.6	ZnSO <sub>4</sub>	87	0.1	0.46
4.1	CoCl <sub>2</sub>	24	—	—
3.9	CoCl <sub>2</sub>	38	Traces	—
3.7	CoCl <sub>2</sub>	52	—	—
3.3	CoCl <sub>2</sub>	66	0.15	—
3.0	CoCl <sub>2</sub>	80	—	0.13

TABLE IVb

*Effect of Metallic ions on Photolysis of β-alanine*TiO<sub>2</sub> added 10 mg

Time of exposure in hrs	Metal ions added	β-alanine in mg	Glycine formed in mg	NH <sub>4</sub> -N in mg
7	—	3.9	0.22	—
7	Co <sup>++</sup>	4.3	—	—
7	Mn <sup>++</sup>	4.3	—	—
7	Mo <sup>++</sup>	4.0	0.20	—
7	Zn <sup>++</sup>	3.9	0.22	—
14	—	3.5	0.35	—
14	Co <sup>++</sup>	4.1	Traces	—
14	Mn <sup>++</sup>	4.1	Traces	—
14	Mo <sup>++</sup>	3.7	0.29	—
14	Zn <sup>++</sup>	3.6	0.40	—
21	—	2.8	0.60	—
21	Co <sup>++</sup>	3.8	0.17	—
21	Mn <sup>++</sup>	3.9	Traces	—
21	Mo <sup>++</sup>	2.8	0.53	—
21	Zn <sup>++</sup>	2.8	0.70	—
28	—	2.5	0.75	—
28	Co <sup>++</sup>	3.8	0.19	—
28	Mn <sup>++</sup>	3.7	0.10	—
28	Mo <sup>++</sup>	2.6	0.60	—
28	Zn <sup>++</sup>	2.5	0.75	—
49	—	0.95	0.65	—
49	Co <sup>++</sup>	2.5	0.13	0.12
49	Mn <sup>++</sup>	2.3	0.10	0.14
49	Mo <sup>++</sup>	1.1	0.41	—
49	Zn <sup>++</sup>	0.9	0.47	0.28

The results presented above, besides pointing out the complexity of the photosensitized reactions of the amino acids also bring into focus the probable significance of these reactions in soil chemical processes.

It has long been recognised that the microbial population in the soil, viz., the bacteria, fungi and actinomycetes are of fundamental importance in the economy of the soil owing to their action in decomposing dead plant remains and in producing compounds such as ammonia and nitrate upon which the new crop is dependent for its growth.

Generally, the compounds left in the soil by plants and added thereto as manure are the nitrogenous compounds such as proteins and their principal degradation products, the amino acids. During the process of decomposition, the nitrogen is released as ammonia. This ammonia is oxidised to nitrite and then to nitrate principally through the agency of the micro-organisms, *Nitrosomonas* and *Nitrobacter*.

Rao and Dhar (1931) have suggested that nitrification in soils must be at least in part a purely photo-chemical process, taking place at the surface of the soil under the influence of sunlight. This claim has been challenged by Waksman and Madhok (1937) who maintain that in normal soils photonitrification is of little practical importance.

In our soil experiments (Giri, Kalyankar and Vaidyanathan, unpublished work) we have observed the production of ammonia from amino acids when their solutions are exposed to sunlight for short periods (21 hrs.) in presence of sterilized soil. However, the intermediate products (e.g., glycine in the case of  $\alpha$ - and  $\beta$ -alanine) could not be detected. This is in confirmation of the evidence presented by Gopala Rao and co-workers (Gopala Rao, 1934; Gopala Rao and Varadaratnam, 1938) to show that ammonification occurs as a purely photo-chemical reaction in the presence of photocatalysts like heated soil,  $TiO_2$  or ignited ferric oxide.

In the light of the results given above it is clear that the failure of some workers (Fraps and Sterges, 1935) to obtain photo-chemical nitrification in presence of sterilized soil can as well be due to the presence in the soil samples of trace elements like manganese which markedly affect the photo-chemical reactions. In this connection our observation that manganese and cobalt ions inhibit the photolysis of amino acids, is noteworthy.

It appears to us, therefore, that the importance of photo-chemical reactions in the chemical processes of the soil is still an open question and should be investigated in detail taking the necessary precautions and under rigorously controlled conditions.

#### SUMMARY

The photolysis of  $\alpha$ - and  $\beta$ -alanine by sunlight in the presence of the sensitizer titanium dioxide has been studied in detail. The glycine formed as an intermediate was estimated by the circular paper chromatographic technique. The effect of pH and the nature of the buffer and the influence of metallic ions on the rate of photolysis were investigated. The significance of these result in soil chemical processes is critically discussed.

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# CHARACTERISATION AND CLASSIFICATION OF SOILS OF THE SUGAR BELT OF BIHAR

## Part VI—Studies on base Constituents

By K. L. KHANNA, B. P. BHATTACHARYA AND SATRUGHAN PRASAD

(*Central Sugarcane Research Station, Pusa, Bihar*)

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### I. INTRODUCTION

Derived molar ratios of the constituents of clay fractions have been employed by Robinson and Holmer (1904), Harrassowitz (1926) and Marbut (1935) in characterising the processes of weathering during soil genesis. From a consideration of the effect of temperature and rainfall on silica-alumina ratio of the clay fraction, Crowther (1930) has derived a measure of leaching which he terms the leaching factor. According to him, well leached soils are characterised by a positive factor while imperfectly leached soils show a negative value. Jenny (1930) on the other hand, has suggested a factor consisting of the ratios of uni and di-valent bases with respect to alumina of the leached horizon and parent material as an index of the loss of bases in percolating waters in soils, and he maintains that the smaller the factor the greater is the leaching of bases with respect to alumina.

The soil divisions in the sugar belt of Bihar have been characterised by Prasad et al (1951), as juvenile soils developed on transported alluvial materials. In the work reported here under an attempt has been made to compare the molar ratios of potash, calcium oxide and magnesia with respect to alumina in the whole soil as also in hydrogen clay from typical profiles representing the important soil divisions with a view to measuring the relative loss of bases with respect to alumina during the process of weathering. Further, a study of the derived ratios along the profile has been employed as a means for assessing the relative translocation of the base constituents which is indicative of the progress of profile development in the soil divisions.

### II. MATERIALS AND METHODS

Procedure adopted for collection of soil samples, separation of hydrogen clay and methods of analysis have been described by Bhattacharya and Prasad in an earlier communication (1954). The results have been expressed on ignited sample basis.

### III. DISCUSSION OF RESULTS

The derived molar ratios of potash and alumina in the whole soil and clay fractions of heavy clay, non-calcareous and calcareous soil divisions as also the differential ratios are presented in Table I.

TABLE I

Molar and differential ratios of Potash and Alumina.

(a)	(b)	(c)	(d)	(e)
Soil divisions	Depth of horizon	Molar $K_2O/Al_2O_3$ ratio in whole soil	Molar $K_2O/Al_2O_3$ ratio in clay fraction	$K_2O/Al_2O_3$ in clay fraction $\frac{K_2O/Al_2O_3 \text{ in clay fraction}}{K_2O/Al_2O_3 \text{ in whole soil}}$
Heavy clay	0—12"	0.26	0.13	0.50
	12"—24"	0.29	0.14	0.48
	24"—36"	0.23	0.15	0.65
Non-calcareous	0—19"	0.40	0.14	0.35
	19"—55"	1.00	0.13	0.13
	55"—72"	0.54	0.15	0.28
Calcareous	0—18"	0.38	0.11	0.30
	18"—40"	0.43	0.21	0.49
	40"—72"	0.49	0.26	0.53

It will be seen that the differential  $K_2O/Al_2O_3$  ratio (column e) in surface horizon shows low values of 0.30 and 0.35 in the calcareous and non-calcareous soils respectively, while in the heavy soil the ratio is wider (0.50). The values of the ratio for the different soil divisions indicate a comparatively higher rate of potash weathering in calcareous and non-calcareous soils as compared to heavy clay. The ratio tends to increase with depth in the calcareous and heavy soil profiles but shows a contrary behaviour in case of non-calcareous soil which also exhibits a markedly low value in the 2nd horizon. The increase in the ratio with depth observed in calcareous and heavy clay soil may be due to less active weathering of potassium in the sub-soil, while the low value obtained with the non-calcareous soil division at a depth of 19"—55" points to a high rate of potash loss immediately below the surface layer during the process of weathering. Molar  $K_2O/Al_2O_3$  ratio (column d) tends to increase along the profile in all the soil divisions indicating the eluviation of potassium from the surface horizons and its mobilisation in the sub-soil. The calcareous soil shows this behaviour to a remarkable degree, the ratio increasing from 0.11 at the surface to 0.26 at 40"—72" as against ranges of 0.13—0.15 and 0.14—0.15 for heavy clay and non-calcareous soil respectively.

The derived and differential molar ratios of calcium oxide and alumina are shown in Table II.

TABLE II

Molar ratios of CaO and Al<sub>2</sub>O<sub>3</sub>

(a)	(b)	(c)	(d)	(e)
Soil division	Depth of horizon	Molar CaO/Al <sub>2</sub> O <sub>3</sub> ratio in whole soil	Molar CaO/Al <sub>2</sub> O <sub>3</sub> ratio in the clay fraction	CaO/Al <sub>2</sub> O <sub>3</sub> in clay fraction $\frac{\text{CaO/Al}_2\text{O}_3}{\text{in whole soil}}$
Heavy clay	0—12"	0.15	0.07	0.466
	12"—24"	0.20	0.09	0.450
	24"—36"	0.24	0.09	0.375
Non-calcareous	0—19"	0.29	0.08	0.280
	19"—55"	0.89	0.10	0.112
	55"—72"	0.49	0.13	0.265
Calcareous	0—18"	2.42	0.08	0.033
	18"—40"	3.35	0.13	0.043
	40"—72"	4.33	0.17	0.040

From a perusal of column *e*, it would appear that the ratio at the surface is as low as 0.03 in calcareous soil as against a high value of 0.28 in the non-calcareous and a very high value of 0.46 in heavy clay, suggesting a higher rate of weathering of calcium in the calcareous soil as compared to the other soil divisions.

From a consideration of the distribution of the ratio along the profile, it will be evident that the rate of weathering of calcium is intense at all depths in calcareous soil while in heavy clay and the non-calcareous soils highest value of the ratio corresponding to the lowest rate of weathering is shown in the surface horizons. The non-calcareous soil, however, shows a low ratio in the depth range of 19"—55" indicating a higher rate of weathering of calcium at this depth.

The molar CaO/Al<sub>2</sub>O<sub>3</sub> ratio (column *d*) in the clay fraction increases with depth in all the soil divisions indicating its translocation from the surface to the sub-soil. The calcareous soil, however, shows an intense leaching of calcium at the surface horizon and a heavy accumulation in the sub-soil (from 0.08 at the surface to 0.17 at 40"—72" depth range).

The derived and differential ratios of magnesia and alumina are presented in Table III.

TABLE III

Molar ratio of MgO and Al<sub>2</sub>O<sub>3</sub>

(a)	(b)	(c)	(d)	(e)
Soil divisions	Depth	Molar MgO/Al <sub>2</sub> O <sub>3</sub> ratio in the whole soil	Molar MgO/Al <sub>2</sub> O <sub>3</sub> ratio in the clay fraction	MgO/Al <sub>2</sub> O <sub>3</sub> in clay fraction MgO/Al <sub>2</sub> O <sub>3</sub> in whole soil
Heavy clay	0—12"	0.91	0.30	0.33
	12"—22"	0.90	0.32	0.36
	24"—36"	0.33	0.18	0.55
Non-calcareous	0—19"	0.76	0.30	0.40
	19"—55"	1.59	0.25	0.16
	55"—72"	0.36	0.21	0.60
Calcareous	0—18"	1.08	0.34	0.31
	18"—40"	1.15	0.38	0.33
	40"—72"	0.33	0.30	0.91

Unlike potash and calcium there is no appreciable difference in respect of this ratio as between the different soil divisions suggesting similar rates of magnesium weathering in all the soil divisions. In the heavy and the calcareous soil divisions, the ratio shows a progressive increase down the profile indicating less intense weathering of this constituent in the sub-soil. The non-calcareous soil, however, exhibits a narrow ratio in the second horizon indicating a higher rate of leaching at this depth, increase in the ratio in the succeeding layer showing less intense leaching of this constituent at lower depths.

The derived MgO/Al<sub>2</sub>O<sub>3</sub> ratio (column *d*) in the clay fraction of all the soil divisions is generally high at the top horizons indicating relative immobility of magnesium as compared to the other bases, *viz.*, calcium and potassium.

The combined and differential ratios of potash and calcium oxide with respect to alumina are shown in Table IV.

TABLE IV

Combined and differential Molar ratios

Soil divisions	Depth of horizon	Molar K <sub>2</sub> O+CaO/Al <sub>2</sub> O <sub>3</sub> in the whole soil	Molar KaO+CaO/Al <sub>2</sub> O <sub>3</sub> in the clay fraction	K <sub>2</sub> O+CaO/Al <sub>2</sub> O <sub>3</sub> in clay fraction K <sub>2</sub> O+CaO/Al <sub>2</sub> O <sub>3</sub> in whole soil
Heavy clay	0—12"	0.419	0.197	0.47
	12"—24"	0.578	0.235	0.41
	24"—36"	0.482	0.343	0.50
Non-calcareous	0—19"	0.687	0.214	0.31
	19"—55"	1.888	0.230	0.12
	55"—72"	1.027	0.273	0.27
Calcareous	0—18"	2.800	0.268	0.10
	18"—40"	3.781	0.343	0.09
	40"—72"	4.827	0.432	0.09



From a perusal of column (e) it is evident that the calcareous soil is characterised by a low differential ratio of the combined bases at the surface horizon as compared to non-calcareous and heavy soils. The low value may be ascribed to the higher rate of weathering of potassium and calcium in the calcareous soil as compared to the other soil divisions. Along the profile, however, the differential ratio is more or less uniform at all depths suggesting uniform rate of weathering in all the soil divisions, the non-calcareous soil being again distinguished by a low value of the ratio in the second horizon.

The combined molar ratio (column d) shows a tendency to increase along the profile indicating translocation of potassium and calcium from the surface to the sub-soil. The calcareous soil, however, shows a very high rate of translocation in as much as the value rises from 0.268 at the surface to 0.432 at a depth of 40"—72".

The combined calcium and magnesium contents as also their differential ratios are presented in Table V.

TABLE V

Combined Calcium and Magnesium contents and differential ratios

(a)	(b)	(c)	(d)	(e)
Soil division	Depth of horizon	Molar CaO+MgO in whole soil	Molar CaO+MgO in clay fraction	$\frac{\text{CaO+MgO in clay fraction}}{\text{CaO+MgO in whole soil}}$
Heavy clay	0—12"	1.06	0.37	0.35
	12"—24"	1.09	0.41	0.38
	24"—36"	0.58	0.27	0.47
Non-calcareous	0—19"	1.05	0.38	0.36
	19"—55"	3.00	0.35	0.12
	55"—72"	0.85	0.34	0.40
Calcareous	0—18"	3.50	0.43	0.12
	18"—40"	4.50	0.44	0.10
	40"—72"	4.66	0.47	0.10

The calcareous soil shows conspicuously low values of the ratio (0.12) at the surface as compared to 0.36 in non-calcareous and 0.35 in heavy clay bringing out clearly the higher rate of weathering of calcium and magnesium in the calcareous soil division. The heavy clay and the non-calcareous soils, however, show higher values down the profile while the calcareous soil indicates a more or less uniform value at all depths. The progressive increase of the ratio down the profile in the heavy clay soil is indicative of lower rate of weathering in the sub-soil; in non-calcareous soil, a heavy loss of the bases is indicated at 19"—55" depth range while in the calcareous soil the weathering is more or less uniform, showing a tendency to increase with depth. The combined molar values (column d) show a more or less uniform distribution of calcium and magnesium along profiles in non-calcareous and calcareous soils while in heavy clay the constituents maintain a slightly higher value in the second horizon, appearing to be stable at a depth range of 12"—24".

#### IV. SUMMARY

The relative rate of weathering and translocation of bases with respect to alumina in the hydrogen clays of the board soil divisions in the sugar belt of Bihar has been employed as an index of their relative maturity.

The higher rate of weathering and translocation of mobile bases like potassium and calcium in the calcareous and non-calcareous soils point to a lower maturity as compared to heavy clay division in which the soil forming processes are comparatively less active.

#### V. ACKNOWLEDGMENT

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# IMPORTANCE OF PHOSPTATES AND OTHER MINERAL CONSTITUENTS OF SOME SOILS

By N. R. DHAR and S. M. BOSE

*Chemical Laboratory, Sheila Dhar Institute of Soil Science, University of  
Allahabad*

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We have carried on a large amount of experimental work on the problem of fixation of atmospheric nitrogen by adding organic matter. Our experimental results show that when finely divided wheat straw or sugar-candy (*Gur*) is mixed with soil and allowed to undergo slow oxidation in air under ordinary temperature and 20.25% moisture, the amount of nitrogen fixed per gram of carbon oxidized is 25 milligrams in light and 11 mgms in the dark, with soil containing 0.04% total nitrogen, 1.0% CaO and 0.079%  $P_2O_5$  (in the hydrochloric acid extract of the soil); whilst with soils richer in calcium phosphate the following results were obtained under similar conditions :—

*Analysis of the soils on oven-dry basis*

TABLE No. 1

		Soil No. I	Soil No. II
1	Total Nitrogen	% 0.208	0.044
2	Silica (Hydrochloric acid insoluble)	% 75.412	76.744
3	Sesquioxides	% 12.240	9.820
4	Iron oxide— $Fe_2O_3$	% 4.350	4.630
	Silica/Sesquioxides ratio	12.030	16.030
5	CaO	% 3.402	2.380
6	MgO	% 0.405	1.859
7	$K_2O$	% 1.139	0.745
8	$P_2O_5$	% 0.418	0.339

The amount of nitrogen fixed in milligrams per gram of carbon oxidized is as follows :

TABLE No. 2

		Soil No. I		Soil No. II	
		Light	Dark	Light	Dark
1	With wheat straw	167.2	116.2	75.6	48.3
2	With sugar-candy ( <i>Gur</i> )	91.2	45.2	44.5	21.7

Hence from the foregoing observations it is clear that when soils rich in calcium phosphate are used, the nitrogen fixations are large. Moreover we have undertaken a systematic analysis of the mineral constituents of different soil samples collected from various fields of the Agriculture Institute Naini, Allahabad, and Sheila Dhar Institute of Soil Science, Allahabad, on which crops are grown every year.

Samples of soil were collected from many places of the field diagonally and thoroughly mixed to form a composite sample from which the final sampling was done by the well-known method of quartering and coning. This sample was dried in an air-oven at 100—105°C for 6 hours and then cooled in a desiccator overnight.

The method followed for the analysis of minerals present in soils were those described in "Soil and Plant Analysis" by C. S. Piper (1947), and also those published by the Association of the Official Agricultural Chemists (1945).

The following results have been obtained.

TABLE No. 3

Percentage composition of Silica and sesquioxides in the first foot and second foot of the soils.

	Percentage composition of			Silica/sesquioxides ratio
	Silica	Sesquioxides	Fe <sub>2</sub> O <sub>3</sub>	
1 Fallow Land				
I First foot of the soil (0—12")	85.638	9.710	5.100	18.5
II second foot of the soil (12"—24")	85.534	10.795	5.205	16.2
2 Sann-Hemp				
Cropped Land				
I First foot	88.094	8.560	4.050	21.3
II Second foot	88.785	7.055	3.650	26.4
3 Pulses				
Cropped Land				
I First foot	81.040	9.790	4.520	17.1
II Second foot	82.254	7.410	3.700	23.2
4 Lucerne				
Cropped Land				
I First foot	87.309	8.281	4.000	21.4
II Second foot	88.125	7.562	3.800	24.0

TABLE No. 4

Percentage composition of calcium as CaO, magnesium as MgO, potassium as K<sub>2</sub>O and phosphorus as P<sub>2</sub>O<sub>5</sub> in the first foot and second foot of the soil.

	CaO	MgO	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
1 Fallow Land				
I First foot	0.6863	0.9668	0.1872	0.1186
II Second foot	0.9339	1.1406	0.2012	0.1242
2 Sann-Hemp				
I First foot	0.3750	0.7930	0.5133	0.1137
II Second foot	0.4125	0.7505	0.5092	0.0982
3 Pulses				
I First foot	3.0100	1.7208	0.5351	0.1117
II Second foot	3.6400	1.6495	0.3976	0.0939
4 Lucerne				
I First foot	2.1248	1.2340	0.5212	0.1128
II Second foot	2.5440	1.1552	0.4028	0.0946

## DISCUSSION

The foregoing results show that the silica-sesquioxides ratio of the soils analysed by us varies from 16.2 to 26.4. Russell has reported a silica-sesquioxides ratio 12.4 in some forest soils. Bear in his book "Soils and Fertilizers" has recorded the analysis of different types of American soil of which the silica-sesquioxides ratio varies from 2.5 to 59.0; whilst that of a clay-loam, silt loam and sandy loam are 15.0, 9.1 and 27.6, respectively. Moreover Raychaudhuri and Mukherjee have obtained very different values varying from 0.193 to 13.1 with different Indian soils.

It is clear from the foregoing results that our ordinary soils are fairly good in their phosphorus content, which is about 0.11 %; whilst the phosphorus content of soils referred in Table No. 1 are fairly high and varies from 0.339 to 0.418%. It is interesting to mention that because of this high phosphorus content of these soils the amount of nitrogen fixation in mgms per gram of carbon oxidized is of a high order.

On the other hand the phosphorus content of the American soils recorded by Bear show a value varying from 0.04 to 0.29%  $P_2O_5$ . Moreover Russell and Voelecker have recorded the following results of Woburn and Rothamsted soils.

Phosphoric acid extracted from Rothamsted and Woburn soils  
(L. A. Deav's data) Mgs  $P_2O_5$ %

	Plot	Inorganic alkali. soluble	Inorganic acid soluble	Total by fusion
Woburn Barley 1927				
No manure	1	40	11	160
Minerals only	4	70	37	250
Minerals and S. Amm.	5a	110	24	250
Farmyard manure	11b	65	23	240
Rothamsted Wheat				
No manure	3	7	40	140
Minerals only	5	9	170	290
Minerals and S. Amm.	8	9	130	260
Farmyard manure	2b	13	130	300

Similarly the  $P_2O_5$  content of the unmanured Danish\* soils is low, i.e., 0.052% and 0.076% respectively for a loam and sandy soils and when farmyard manure is added, it goes up to 0.064% and 0.084% respectively. It is also interesting to record here the following lines from "Soil Conditions and Plant Growth," by Russell.

"Phosphorus is peculiar in that some soils are notably deficient in it: these occur commonly in Australia and South Africa, but also in the United Kingdom, in consequence the vegetation is affected and the grazing animals suffer serious phosphate starvation."

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\* Forsg med. Staldgodning og Kunstgodning Ved Askov, 1894—1948.  
Ved Karsten Iverson og K. Dorph.—Peterson.

Moreover our results show that the silica-sesquioxides ratio of our alluvial soils are higher than in most cold country soils.

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## IMPORTANCE OF CALCIUM PHOSPHATE STATUS OF SOILS IN RELATION TO THE LOSS OF LIME BY LEACHING

By N. R. DHAR and S. G. MISRA

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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It is well known that calcium carbonate, magnesium carbonate, calcium oxide, magnesium oxide, limestone, marl, dolomite, etc. are used as liming materials in industrialized countries. As the first and second dissociation constants of carbonic acid are small, as soon as carbonate ions from the liming material come in contact with the hydrogen ions, undissociated carbonic acid and bicarbonate ions are readily formed and the acidity of the system decreases. In this process, a good deal of calcium and magnesium compound is washed away by rain.

A large amount of work on the leaching of these plant food materials by rains has been carried on in different parts of the world. Ten year results, from Cornell University lysimeters, secured by Bizzell and Lyon<sup>1</sup> show an average annual loss of 995 pounds of calcium carbonate per acre from the bare soil and 535 pounds from plots that grew crops. In North Central States of America the annual loss of lime ranges between 100—500 pounds per acre. The Illinois Station concludes that 540—760 pounds of calcium carbonate is leached away from the surface 21 inches of the soil annually. Some of the results<sup>2</sup> obtained regarding the loss of lime in other places are recorded below :—

<i>Loss of CaO in pounds per acre per annum</i>	
<i>(Drainage water from unmanured plots)</i>	
Rothamsted	330.0
Aberdeen	76.0
Ithaca (New York States)	367.0
Florida	140.0

The Göttingen fields in Germany show a maximum loss of 206 pounds of CaO and 52.0 pounds of MgO per acre whilst the minimum losses are 175.8 pounds and 3.0 pounds respectively. The loss of magnesia from the Rothamsted fields' drainage water ranges from 5.7 to 8.8 pounds per acre. In Aberdeen 30—35 pounds of MgO and in California<sup>3</sup> even 181 pounds of MgO per acre have been found to be present in water solutions from the cropped soils. The poorer the soil in lime, the smaller is the loss. In this connection the following lines from Russells' "Soil Conditions and Plant Growth," 1917, p. 47, are of interest :—

"Calcium carbonate is not a permanent constituent of the soil but changes into calcium bicarbonate and washes into the drainage water. The average loss per acre per annum throughout England and Wales has been estimated at 500 pounds and at Rothamsted on the arable land at 800—1000 pounds. The rate of loss is influenced by treatment: being increased by the use of ammonium sulphate and decreased by dung and crops—it is much less on pastures than on arable land. Repeated additions of calcium carbonate are, therefore, necessary."

The humus store of the soils has been reported by some workers to act as a buffer and reduce the leaching of lime due to its high adsorption by the humus colloids and its being resistant to leaching. In a recent paper Dhar<sup>4</sup> has emphasized that the addition of phosphates to soils leads to the preservation of calcium carbonate chiefly because in normal soils dicalcium phosphate ( $\text{CaHPO}_4$ ) is readily formed when the soil is rich in calcium carbonate and to which a phosphate is added. Moreover, the solubility of dicalcium phosphate ( $\text{CaHPO}_4$ ) being much less than that of calcium bicarbonate,  $\text{Ca}(\text{HCO}_3)_2$ , which is also formed in the soil, considerably saves the leaching of lime. Also, phosphate rich soils are high in their nitrogen contents hence it is necessary to increase the calcium phosphate reserve of the soils all over the world for the maintenance and improvement of their fertility.

Experiments were carried on in order to find out whether a high humus and calcium phosphate status of the soil can contribute largely to the saving of lime by leaching because it is the general impoverishment of the soil exchange complex in calcium ions which gives rise to the acid soils.

#### *Experimental Procedure and Results*

Known weights of the soils were shaken in glass stoppered bottles with measured volumes of distilled water for two hours and calcium and magnesium in the filtrates were determined after an interval of 48 hours. Distilled water was again added, the volume was made up and the process was repeated. In this way the total calcium and magnesium in these washings was found out and the average was taken. Calculations were made in pounds per acre of the soil up to 9 inches depth considering the weight of soil to be 1000 tons per acre. Dilute salt solutions of sodium chloride, potassium chloride, sodium sulphate and potassium sulphate of 0.01 molar concentration were also used for washing the same soils repeatedly and the amounts of lime and magnesia were determined in the filtrates. The average loss of  $\text{CaO}$  and  $\text{MgO}$  per washing was calculated in pounds per acre of the soil and has been reported.

The soils used in this experiments have been designated as good soil, garden soil and normal soil. The samples of these soils were taken out from the surface 9 inches of the fields, were powdered and then passed through a 100 mesh sieve. Subsequent analyses were made on the basis of the oven-dried soils. The good quality soil is rich in its humus and calcium phosphate content, the garden soil shows an intermediate behaviour whilst the normal soil is poor in humus and calcium phosphate status. The chemical analyses of these soils is recorded below :—

#### Per cent. Chemical Composition (oven-dry basis)

	Good soil	Garden soil	Normal soil
Loss on ignition	8.800	3.810	3.880
HCl-insoluble	74.220	79.940	81.640
Sesquioxides	9.830	9.860	11.170
$\text{Fe}_2\text{O}_3$	4.350	4.260	4.120
$\text{CaO}$	4.080	2.800	0.992
$\text{MgO}$	1.770	1.730	1.158
$\text{K}_2\text{O}$	0.995	0.987	0.735
$\text{P}_2\text{O}_5$	0.418	0.121	0.079
pH	7.4	8.0	7.6
Total Nitrogen	0.249	0.076	0.041
Total Carbon	1.659	0.670	0.400
Total Carbonates (as $\text{CaCO}_3$ )	4.640	3.950	1.240
Available $\text{P}_2\text{O}_5$ (1% citric acid)	0.128	0.020	0.0216



The amount of lime and magnesia in pounds per acre per washing with distilled water and salt solutions has been given in the following tables separately in order to compare the losses. The soil water ratios at which the losses have been calculated is also recorded. However, the losses with dilute salt solutions have been calculated only at 1:2 soil solution ratio. The average of the number of washings has also been given :

TABLE No. 6

Loss of Lime and Magnesia in lbs/acre per washing  
(with Distilled water)

		Soil-water ratios			
		1:2	1:3	1:5	1:10
		Average of 18 washings		Average of 28 washings	
Good soil	{ CaO	222.0	224.0	284.0	371.0
	{ MgO	67.0	67.0	67.0	68.5
Normal soil	{ CaO	185.4	...	...	264.3
	{ MgO	9.0	...	...	9.9

(With Salt Solutions)

		NaCl	KCl	Na <sub>2</sub> SO <sub>4</sub>	K <sub>2</sub> SO <sub>4</sub>
		Average of 20 Washings			
Good soil	{ CaO	468.8	403.2	667.5	741.4
	{ MgO	88.2	72.7	82.9	71.7
Garden soil	{ CaO	627.2	658.5	795.0	873.6
	{ MgO	71.1	67.3	78.4	67.2
Normal soil	{ CaO	591.3	297.9	800.0	1232.0
	{ MgO	34.0	32.0	31.3	31.3

The loss of lime in different washings has been recorded in the following table in order to compare the losses when good soil and normal soil are washed with distilled water at a higher soil-water ratio.

Loss of Lime in different washings with water  
(lbs/acre)

Washings	Good soil		Normal soil	
	1:50	1:25	1:25	
1st	1906.4	1505.0	1317.0	
7th	1858.0	...	683.6	
10th	1176.0	940.8	...	
17th	952.0	664.8	584.6	

Discussion

From the foregoing tables it is clear that greater amounts of lime and magnesia are washed out with salt solutions than with distilled water from the soils. Moreover, the amounts of lime (CaO) leached from the good quality soil with distilled water are greater than the normal soil containing smaller amounts of lime. The amounts leached, also, steadily falls off as the washing is continued. The amount of calcium oxide and magnesium oxide leached with ratios of soil to distilled water having the value 1:2 or 1:3 are of the same order as obtained in the Rothamsted fields with rain water.

It is also clear that the good quality soil which is rich in calcium phosphate and lime, loses less calcium oxide when washed with dilute solutions of sodium chloride, potassium chloride, sodium sulphate and potassium sulphate than the garden soil which is much less rich in calcium phosphate and lime. The normal soil which is poor in calcium phosphate loses more lime than the good quality soil when washed with the same salt solutions. On the other hand, when these soils are washed with distilled water, in general, the good quality soil loses more lime than the normal soil. But the ratio of the lime contents of good soil and the normal soil is about 4:1 whilst the ratio of calcium lost by washing is approximately 3:2 (at soil-water ratios of 1:2 and 1:10). The ratio of their phosphate contents is about 5:1. It appears, therefore, that the richness of the good quality soil regarding calcium phosphate may be preventing its loss of lime on leaching.

It is interesting to record here that if the amount of phosphate present in the good quality soil is calculated as  $\text{Ca}_3(\text{PO}_4)_2$ , it comes to 20440 lbs/acre up to 9 inches depth of the soil (=9363 lbs/acre of  $\text{P}_2\text{O}_5$ ). From our washing experiments it seems that such a phosphate rich soil, even when washed with rain water at the rate of 25 inches per year (about 1:2.5 soil-water ratio), changes its pH from 7.4 to 7.3 in approximately 500 years whilst the change of pH to acidity, i.e., pH of 6.4 requires washing for more than 2000 years. On the other hand, the calcium phosphate poor normal soil containing 3861.4 lbs of  $\text{Ca}_3(\text{PO}_4)_2$  per acre (=1769 lbs/acre  $\text{P}_2\text{O}_5$ ) when washed with rain water at the rate of 25 inches per year should change its pH from 7.6 to 7.3 in 45 years whilst it becomes acidic with a pH of 6.4 when washed with rain water for 450 years.

This clearly shows that phosphate rich soils have much less tendency to be acidic than the calcium phosphate poor soils in leaching by rain-water. Hence the calcium phosphate reserve of world soils must be improved to avoid acidity and to help nitrogen fixation by the addition of organic matter to such soils as has been demonstrated by Dhar and coworkers at Allahabad.

Moreover, our result on the washing away of calcium and magnesium by different soils containing varying amounts of calcium phosphate clearly show that land fertility lasts for larger time in calcium phosphate rich soils than in calcium phosphate poor soils. Our results and conclusions are in agreement with the important observations made by O. Arrhenius<sup>5</sup> showing that soils near human habitations, past and present, in different parts of Sweden and Java are rich in phosphate and fertile. Similar results have been recorded in the neighbourhood of cities in Denmark. It has also been reported in U.S.A. that the areas of soil richest in phosphorus occur in the limestone belt of Kentucky where soils are known to contain well over 10 thousand pounds per acre of phosphorus and these lands are highly fertile and are maintaining fertility for a long time.

The beneficial effect of phosphates on the saving of lime can be very well explained from solubility point of view. It is well-known that carbonic acid is much weaker acid than phosphoric acid as their dissociation constants show<sup>6</sup> :—

	Dissociation constant	First dissociation constant	Second dissociation constant	Third dissociation constant
$\text{H}_3\text{PO}_4$	$9 \times 10^{-3}$	$1.1 \times 10^{-2}$	$2 \times 10^{-7}$	$3.6 \times 10^{-13}$
$\text{H}_2\text{CO}_3$	$3 \times 10^{-7}$	$3 \times 10^{-7}$	$6 \times 10^{-11}$	...

As the dissociation constant of carbonic acid is much smaller than that of phosphoric acid, calcium carbonate is much more alkaline than tricalcium phosphate although their water solubilities are the same at 0°C. Because the third

dissociation constant of phosphoric acid is smaller than the first and second dissociation constants of carbonic acid the possibility of formation of  $\text{CaHPO}_4$  in the soil from calcium phosphate and hydrogen ions is greater than the formation of  $\text{Ca}(\text{HCO}_3)_2$  from calcium carbonate and hydrogen ions and this is of very great importance in saving lime and phosphate in soils. Hence whenever hydrogen ions are available in soil solution along with phosphate ions,  $\text{HPO}_4^{11}$  ions are more readily formed than  $\text{HCO}_3$  ions and as  $\text{CaHPO}_4$  is much less soluble than  $\text{Ca}(\text{HCO}_3)_2$ , the loss of time by leaching is greatly reduced. The solubility data clearly explain this point :—

Solubilities of Calcium carbonate and Calcium phosphates in gms/litre at  $0^\circ\text{C}$

$\text{CaCO}_3$	0.0013
$\text{Ca}_3(\text{PO}_4)_2$	0.0013
$\text{CaHPO}_4$	0.028
$\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	4.000 at $15^\circ\text{C}$

Thus calcium phosphate reserve of the soil not only acts as an excellent buffer but it saves lime and also being less alkaline than calcium carbonate oxidizes the humus present in the soil only very slowly and thus the loss of humus is less than with calcium carbonate. Hence, the humus status of a soil also improves in the presence of high calcium phosphate content. Also, as  $\text{CaHPO}_4$ , produced from  $\text{Ca}_3(\text{PO}_4)_2$  in the long run is sure to create more alkalinity because it is not washed out so readily as calcium bicarbonate hence the lime requirement of a soil decreases, i.e., the pH of the soil does not fall off.

*Role of soluble phosphates in saving Lime*

Some experiments have been performed by taking 25 grams of the soils, passed through 100 mesh sieve, and 150 c. c. solution of the soluble phosphates of sodium of varying concentrations from M/100 to M/5000 in glass stoppered bottles. The contents were shaken for 2 hours and then filtered after 2½ hours contact at room temperature. The filtration was done in Buchner funnel with a filter paper fitted in a filtering flask which was connected to a filter pump. The soil mass on the Buchner funnel was separated and then shaken for 2 hours with 150 c. c. of distilled water and calcium was determined in the filtrate after 24 hours. In this way 8 washings were made and the amount of calcium in the filtrates was determined each time. Dilute solutions of monocalcium phosphate and superphosphate were also used and then the calcium was determined in the water extracts of the treated soils as described previously. All the soils were washed with distilled water, without being previously treated with any phosphate in order to compare the relative retardation of loss of lime from phosphated soils.

Amount of CaO given out per 100 gms of soils

(a) Soils without any treatment  
(Distilled water alone)

No of Washings	Amount of CaO given out per 100 gms of soils		
	Good soil	Garden soil	Normal soil
1	0.0443	0.0181	0.0235
2	0.0392	0.0179	0.0207
3	0.0382	0.0175	0.0204
4	0.0369	0.0157	0.0197
5	0.0425	0.0179	0.0224
6	...	0.0174	0.0190
7	0.0358	0.0324	0.0313
8	0.0280	0.0200	0.0200

(b) After treating the soils with Disodium hydrogen phosphate

Good soil

Amount of CaO given out per 100 gms of soil

No. of washing with water	Concentration of $\text{Na}_2\text{HPO}_4$ for treating the soil			
	M/100	M/500	M/1000	M/5000
1	0.0089	0.0213	0.0213	0.0244
2	0.0207	0.0230	0.0369	0.0257
3	0.0199	0.0240	0.0257	0.0260
4	0.0347	0.0246	0.0291	0.0280
5	0.0420	0.0302	0.0320	0.0280
6	0.0443	0.0268	0.0246	0.0230
7	0.0380	0.0268	0.0257	0.0240
8	0.0329	0.0360	0.0400	...

Garden soil

1	0.0045	0.0089	0.0095	0.0145
2	0.0060	0.0134	0.0134	0.0168
3	0.0060	0.0123	0.0133	0.0168
4	0.0105	0.0134	0.0123	0.0179
5	0.0139	...	0.0168	0.0224
6	0.0145	0.0179	0.0168	...
7	0.0175	0.0179	0.045	0.0168
8	...	...	...	0.0168

Normal soil

1	0.0035	0.0078	0.0112	0.0123
2	0.0067	0.0157	0.0157	0.0179
3	0.0065	0.0179	0.0179	---
4	0.0150	0.0168	0.0170	0.0246
5	0.0161	---	---	0.0313
6	0.0224	0.0179	0.0168	0.0213
7	0.0201	0.0179	0.0201	0.0201
8	0.0300	0.0300	---	---

(c) After treating the soils with Monosodium dihydrogen phosphate of M/100 concentration

No. of Washings	Good soil	Garden soil	Normal soil
1	0.0201	0.0168	0.0145
2	0.0257	0.0165	0.0179
3	0.0201	0.0123	0.0168
4	---	---	0.0201
5	0.0235	0.0168	0.0201
6	0.0235	0.0190	0.0179
7	0.0270	0.0285	0.0235
8	0.0285	---	0.0257

(d) After treating the soils with Monocalcium phosphate of varying concentrations

No. of Washing	Good soil			Garden soil			Normal soil		
	M/100	M/500	M/1000	M/100	M/500	M/1000	M/1000	M/500	M/1000
1	0.0392	0.0302	0.0302	0.0358	0.0213	0.0213	0.0390	0.0314	0.0199
2	0.0392	0.0302	0.0290	0.0325	0.0210	0.0145	0.0318	0.0224	0.0190
3	0.0331	0.0392	0.0302	0.0302	0.0212	0.0157	0.0257	0.0222	0.0201
4	0.0330	0.0358	0.0336	0.0280	0.0168	0.0157	0.0224	0.0280	0.0200
5	0.0267	0.0314	0.0257	0.0196	0.0123	0.0146	0.0201	0.0157	0.0157
6	0.0290	0.0269	0.0257	0.0170	0.0157	0.0190	0.0159	0.0179	0.0157
7	0.0330	0.0246	0.0327	0.0190	0.0123	0.0212	0.0190	0.0134	0.0190
8	0.0336	0.0257	0.0246	0.0200	0.0168	0.0200	0.0224	0.0146	0.0180

(e) After treating the soils with Superphosphate at the rate of 150 lbs.  $P_2O_5$  acre of soil

No. of Washings	Good soil	Garden soil	Normal soil
1	0.0347	0.0150	0.0190
2	0.0302	0.0146	0.0190
3	0.0280	0.0157	0.0200
4	0.0291	0.0146	0.0224
5	0.0369	0.0201	0.0224
6	0.0246	0.0174	0.0190
7	0.0392	0.0207	0.0213
8	0.0280	0.0230	0.0213

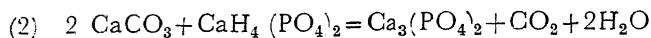
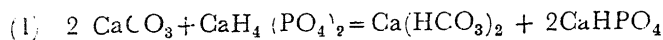
It is quite clear from the above tables that after treating the soil with phosphate at higher concentrations the loss of lime decreases markedly in all the soils but at lower concentrations this effect gradually decreases. M/5000 concentration of disodium hydrogen phosphate corresponds to about 79.0 lbs of  $P_2O_5$  per acre which is the dose generally applied to the fields as phosphate fertilizers. Monosodium dihydrogen phosphate treatment to the soils checks the loss of lime but to a lesser degree in comparison to disodium hydrogen phosphate at M/100 concentration. Even superphosphate, at the rate of 150 lbs of  $P_2O_5$  per acre when added to the soil reduces the loss of lime. The behaviour of monocalcium treated soils is, however, different. Higher concentrations of it increase the loss of lime in subsequent washings with distilled water except in the good quality soil. This loss decreases as the concentration of monocalcium phosphate used for treating the soil is lowered.

The beneficial effect of soluble phosphates may be ascribed to the combination of soluble calcium ions with the phosphate ions to form the sparingly soluble tricalcium and dicalcium phosphates. Thus the calcium and magnesium ions which were in soluble condition become insoluble and hence when these phosphated soils are further washed with distilled water the calcium ions coming into solution became smaller.

The addition of small doses of monocalcium phosphate or superphosphate leads to the preservation of calcium in the soil which otherwise might have been washed away as  $Ca(HCO_3)_2$ ,  $CaSO_4$  and  $Ca(NO_3)_2$ . Moreover,

the tricalcium and dicalcium phosphates formed from the calcium carbonate of the soil by the action of monocalcium phosphate are sure to create more alkalinity in the long run because dicalcium phosphate is not washed away as readily as calcium carbonate. Also, as the second dissociation constant of phosphoric acid is greater than that of carbonic acid hence the formation of monocalcium phosphate from tricalcium and dicalcium phosphate is less pronounced than the formation and washing away of calcium bicarbonate from calcium carbonate and hydrogen ions.

At higher doses the behaviour of monocalcium phosphate is quite different and a greater leaching of calcium seems to take place. The monocalcium phosphate can react in two ways with calcium carbonate of the soil :—



It is clear, therefore, that the first reaction is prominent because greater loss of lime can take place.

*Tata basic slag. Dicalcium phosphate and Tricalcium phosphate also check the loss of lime on leaching*

1.25 grams of finely powdered (150 mesh) Tata basic slag, dicalcium phosphate and tricalcium phosphate were added to 25 grams of the soils and the mixture was shaken in glass stoppered bottles for 2 hours with 150 c.cs. of distilled water. After 24 hours the filtrates were analysed for their calcium contents. To these washed soil samples 150 c.cs. of distilled water was again added and the process was repeated several times. A separate control experiment was carried out by taking 1.25 grams of basic slag or dicalcium phosphate or tricalcium phosphate and 150 c. cs. of distilled water. Calcium in the filtrates was determined after 24 hours. The amounts of calcium oxide in the various filtrates of the mixture of soil plus phosphate material and phosphate alone have been recorded in the following tables :—

(a) 25 gms Soils + 1.25 gms Tata Basic Slag  
Calcium oxide given out per 25 gms of soils

No of Washings	1.25 gms slag + 150 c. c. water	Good soil		Garden soil		Normal soil	
		Soil+basic slag	Soil alone	Soil+basic slag	Soil alone	Soil+basic slag	Soil alone
1	0.0120	0.0201	0.0111	0.0131	0.0045	0.0140	0.0059
2	0.0106	0.0115	0.0098	0.0096	0.0045	0.0106	0.0052
3	0.0064	0.0098	0.0095	0.0101	0.0046	0.0092	0.0051
4	0.0067	0.0109	0.0092	0.0103	0.0039	0.0092	0.0049
5	0.0053	0.0078	0.0106	0.0078	0.0045	0.0070	0.0056
6	0.0075	0.0081	—	0.0078	0.0043	0.0075	0.0047
7	0.0098	0.0073	0.0089	0.0078	0.0081	0.0078	0.0078
8	0.0098	0.0101	0.0070	0.0101	0.0050	0.0100	0.0050

(b) 25 gms Soil + 1.25 gm Dicalcium phosphate ( $\text{CaHPO}_4$ )  
Calcium oxide given out per 25 gms of soils

No. of Washings	1.25 gm $\text{CaHPO}_4$ + 150 c. c. water	Good soil		Garden soil		Normal Soil	
		Soil + $\text{CaHPO}_4$	Soil alone	Soil + $\text{CaHPO}_4$	Soil alone	Soil + $\text{CaHPO}_4$	Soil alone
1	0.0045	0.0100	0.0111	0.0045	0.0045	0.0056	0.0059
2	0.0039	0.0075	0.0098	0.0045	0.0045	0.0050	0.0052
3	0.0039	0.0059	0.0095	0.0046	0.0046	0.0045	0.0051
4	0.0039	0.0056	0.0092	0.0039	0.0039	0.0047	0.0049
5	0.0040	0.0064	0.0106	0.0036	0.0045	0.0045	0.0056
6	0.0042	0.0067	—	0.0036	0.0043	0.0056	0.0047
7	0.0042	0.0084	0.0089	0.0036	0.0081	0.0056	0.0078
8	0.0040	0.0084	0.0070	0.0036	0.0050	0.0056	0.0050

(c) 25 gms Soil + 1.25 gms Tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ )

No. of Washings	1.25 gms $\text{Ca}_3(\text{PO}_4)_2$ + 150 c. c. water	Good soil		Garden soil		Normal soil	
		Soil + $\text{Ca}_3(\text{PO}_4)_2$	Soil alone	Soil + $\text{Ca}_3(\text{PO}_4)_2$	Soil alone	Soil + $\text{Ca}_3(\text{PO}_4)_2$	Soil alone
1	0.0092	0.0103	0.0111	0.0059	0.0045	0.0078	0.0059
2	0.0067	0.0064	0.0098	0.0047	0.0045	0.0050	0.0052
3	0.0064	0.0059	0.0095	0.0056	0.0046	0.0059	0.0051
4	0.0056	0.0056	0.0092	0.0043	0.0039	0.0047	0.0049
5	0.0056	0.0056	0.0106	0.0042	0.0045	0.0050	0.0056
6	0.0050	0.0059	—	0.0042	0.0043	0.0042	0.0047
7	0.0047	0.0067	0.0089	0.0050	0.0081	0.0047	0.0078
8	0.0036	0.0067	0.0070	0.0050	0.0050	0.0064	0.0050

From the foregoing tables it is clear that the sum of calcium ions in the filtrates of soils alone and basic slag alone is greater than the amount of calcium ions in the filtrates of their mixtures. Thus the saving of lime takes place on washing. This behaviour is quite marked in good soil. With dicalcium phosphate the saving of lime is appreciable in good soil, there being practically no saving in garden soil and a little saving in the case of normal soil if the amount of calcium ions in the filtrates of dicalcium phosphate is not taken into account. Similar results are obtained with tricalcium phosphate. However, if we take into account the amount of lime dissolved out from the di and tricalcium phosphates there is a definite saving of lime in each soil on leaching. Thus even these sparingly soluble phosphates help in saving lime from leaching losses because they appreciably hydrolyse in water and give rise to phosphate ions which combine with the calcium ions in solution to form insoluble compounds like  $\text{CaHPO}_4$ .

*Alkali soil formation and the Calcium phosphate status of soils*

When the soils, used in these experiments, are washed repeatedly with dilute salt solutions of sodium chloride, potassium chloride, sodium sulphate and potassium sulphate of 0.01 M concentrations, an alkaline reaction is developed due to replacement of calcium ions by the sodium or potassium ions from dilute solutions of sodium or potassium salts by which the soils have been washed. As it is evident from the following table the good quality soil, rich in humus and calcium phosphate is least alkaline when washed with the above salt solutions.

The normal and garden soils being poor in humus and calcium phosphate attain higher pH value on being washed repeatedly with same salt solutions.

#### Effects of leaching with Salt solutions

Soils				pH after washing with salt solutions			
	Total C %	Total P O <sub>5</sub> %	Original pH	NaCl	KCl	Na <sub>2</sub> SO <sub>4</sub>	K <sub>2</sub> SO <sub>4</sub>
Good soil	1.659	0.418	7.4	8.2	7.8	7.9	7.9
Garden soil	0.670	0.121	8.0	8.5	8.4	8.6	8.7
Normal soil	0.400	0.079	7.6	8.5	8.1	8.2	8.3

Hence the soils that are rich in humus and calcium phosphate are not readily converted into alkaline soils whilst the soils poor in humus and calcium phosphate have a marked tendency to be alkaline when washed by saline waters containing sodium or potassium salts.

#### SUMMARY

Careful experiments on the washing of calcium by distilled water from different soils show clearly that the pH values of the soil falls off when washing is continued. Moreover the falling off of pH values is more pronounced with soil poor in humus and calcium phosphate.

A good quality soil containing 0.42% P<sub>2</sub>O<sub>5</sub> when washed with rain water at the rate of 25 inches per year changes its pH value from 7.4 to 7.3 in 500 years whilst the change of pH to 6.4 requires washing for more than 2000 years. Another soil containing 0.079% P<sub>2</sub>O<sub>5</sub> changes its pH from 7.6 to 7.3 in 45 years only.

Greater amounts of calcium ions are washed out by the dilute solutions of NaCl, KCl, Na<sub>2</sub>SO<sub>4</sub>, and K<sub>2</sub>SO<sub>4</sub> than by distilled water alone. The same good quality soil liberates smaller amounts of calcium ions when compared to other soils less rich in calcium phosphate although the calcium content of good soil is quite light. This is certainly due to the high calcium phosphate status of good quality soil that saving of lime takes place on leaching with water or salt solutions.

When soils are treated with dilute solutions of Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>, H<sub>2</sub>O and superphosphate the possibility of washing away of lime from such treated soils decreases markedly.

Similarly, soils when treated with finely powdered Tata basic slag, dicalcium phosphate and tricalcium phosphate suffer less loss of lime on leaching.

A high calcium phosphate status of soils resists the conversion of a normal soil to alkaline soil by leaching with salt solutions.

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# EFFECT OF POTASSIUM CHLORIDE AND CALCIUM CHLORIDE ON NITRIFICATION AND NITROGEN LOSS FROM SOLUTIONS OF DIFFERENT NITROGEN RICH COMPOUNDS

By N. R. DHAR and S. K. GHOSH

*Chemical Laboratory, Sheila Dhar Institute of Soil Science, University of Allahabad*

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It has been emphasised in a number of publications <sup>1, 2</sup> from this laboratory that light plays an important part in many oxidation reactions and the process of ammonification, nitrification and loss of nitrogen are mainly surface reactions and markedly accelerated by light absorption.

We have carried on further experiments on nitrogen loss and nitrification of a number of nitrogenous compounds in contact with chemically pure solid surface in artificial light and in the dark and the effect of potassium chloride and calcium chloride on them. The experiments have been carried on both under sterile and unsterile conditions.

## EXPERIMENTAL

Soluble nitrogenous compounds of A. R. quality were used and the solid surfaces used were also of Merck's reagent. The reaction mixtures were taken in Jena glass test-tubes. For unsterile experiments, in all cases the total volume of the solution in the test-tubes was kept to 5 mls, which was made up by adding distilled water where necessary. Two such sets of test-tubes plugged with cotton wool were arranged. One set was carefully covered with thick black cloth and the other set was hung in front of a 1000 watt tungsten filament bulb. Both the light and the dark sets were kept in a wooden chamber and placed approximately at a distance of  $2\frac{1}{2}$  ft. from the bulb. In order to avoid a large rise of temperature, an electric fan was worked from behind so that the temperature variation inside the chamber was within  $\pm 2^\circ\text{C}$ . The light source was used only during laboratory hours at the average of 6 to 8 hours each day.

Similar arrangements were also made for sterile experiments. Sterilisation in all cases was carried out in an autoclave at 10 lbs pressure for half an hour, and in such cases, the volume of the solutions in test-tubes was maintained at 10 mls before sterilisation. One set of test-tubes containing the reaction mixtures was exposed to light from a 60 watt electric bulb whilst the other similar set was covered with thick black cloth. Both the sets were kept at the same place at a distance of  $2\frac{1}{2}$  ft. from the bulb. The exposure was continued day and night and the test-tubes occasionally shaken to facilitate aeration. The average mean temperature was recorded during the exposure.

In all these experiments (both unsterile and sterile), ammoniacal, nitric and total nitrogen were estimated at different intervals of time. For the purpose of these estimations, the total amount of the solid and the solution in the test-tubes was carefully leached with a 0.05N potassium chloride solution so that any adsorbed nitrogenous cation or anion may be completely displaced. The leachate was made up to a known volume and an aliquot part analysed for ammoniacal and nitric nitrogen. For the estimation of total nitrogen an aliquot part of the leachate was evaporated to dryness in a Kjeldahl flask with a drop of concentrated sulphuric acid and the nitrogen estimated by salicylic acid reduction method.<sup>3</sup>

Results from tables 1 to 3 show that there is a loss of nitrogen from ammonium salts and urea when they are allowed to undergo oxidation on different solid surfaces. In the case of ammonium salts, the nitrogenous compounds which have not undergone loss are present mostly in available form. This available form of nitrogen, which is obtained by the summation of ammoniacal and nitric nitrogen, equals the total nitrogen estimated separately with experimental error. For urea, however, there is no nitrogen in the available form in the beginning, but both, ammoniacal and nitric nitrogen, appear after this nitrogenous compound remains in contact with various solid surfaces for some time. It is clear, therefore, that when nitrogenous compounds like ammonium chloride, ammonium sulphate and urea used in our experiments undergo nitrification, then along with the loss of nitrogen, a certain fraction of them is converted into nitrate nitrogen. The efficiency of nitrate formation now requires a critical examination. In the case of ammonium salts, a certain fraction of the ammoniacal, nitrogen decreases, which is obtained by the difference in the ammonia content before and after the experiment. Some of this ammoniacal nitrogen, during the nitrification, is converted into nitric nitrogen, and the efficiency of the nitrate formation has been calculated from the amount of nitric nitrogen formed per 100 grams of this ammoniacal nitrogen which has decreased from the system. In the case of urea, however, there is no ammoniacal nitrogen to start with, but it accumulates in the system after some time, and hence, in this case, the efficiency of nitrate formation has been calculated from the amount of nitric nitrogen present per 100 grams of the available nitrogen obtained from urea. In table Nos. 4 to 9 results recording the percentage loss of nitrogen and the efficiency of nitrate formation from various nitrogen rich compounds in contact with different solid surfaces are given.

A perusal of tables 4 to 9 show that with all solid surfaces used in these experiments there is a loss of nitrogen from the nitrogenous compounds to different extents. It is, however, apparent that the loss of nitrogen is always more prominent in those systems which are exposed to light. Amongst all the surfaces zinc oxide surface seems to be more pronounced in causing nitrogen loss specially from ammonium salts. Again the presence of potassium chloride checks the loss of nitrogen and that greater the concentration of potassium chloride added, smaller is the loss of nitrogen from nitrogenous compounds.

Another significant point which is brought out from these results is that the percentage conversion of nitrate from the oxidation of ammonium salts is, in general, more marked in dark than in the light and the presence of potassium chloride considerably enhances the nitrate formation. Again it will be seen from these results that in presence of zinc oxide the loss of nitrogen from ammonium sulphate is very large but the percentage of nitrate formation is comparatively small. With barium sulphate surface where the loss of nitrogen from ammonium salts is little, there is in general, more nitrate formation. Also, in the case of urea, the percentage of nitrate formation is more pronounced with barium sulphate surface where the loss of nitrogen is less marked than with calcium phosphate surface where the loss of nitrogen is greater during the same interval of time. It is also observed that the effect of potassium chloride in enhancing nitrate formation is more marked in the dark where the loss of nitrogen practically in all cases is less than in light.

We have also carried on these experiments under completely sterile condition to exclude bacterial action during nitrate formation and nitrogen loss. In some of these experiments instead of potassium chloride the influence of calcium chloride has been investigated. The experimental results are recorded in Tables 10 to 18

TABLE No. 1

		EXPERIMENTS WITH AMMONIUM CHLORIDE AND POTASSIUM CHLORIDE				Average temperature = 40°C		
Treatment	Original amounts present in grams	Amounts obtained in contact with Fe <sub>2</sub> O <sub>3</sub> surface after 75 days		Amounts obtained in contact with Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 180 days		Amounts obtained in contact with BaSO <sub>4</sub> surface after 145 days		
		Exposed to light for 382 hrs	Kept in dark	Exposed to light for 768 hrs	Kept in dark	Exposed to light for 618 hrs	Kept in dark	
1 gm surface + 0.1 gm N as NH <sub>4</sub> Cl	NH <sub>3</sub> —N	0.100	0.08866	0.09025	0.09076	0.09289	0.09036	0.09246
	NO <sub>3</sub> —N	...	0.00515	0.00501	0.00235	0.00202	0.00485	0.00455
	Available—N	0.100	0.09381	0.09526	0.09311	0.09491	0.09521	0.09701
	Total—N	0.100	0.09392	0.09532	0.09320	0.09507	0.09526	0.09710
1 gm surface + 0.1 gm N as NH <sub>4</sub> Cl + 0.5 gm K as KCl	NH <sub>3</sub> —N	0.100	0.09306	0.09459	0.09288	0.09516	0.09336	0.09525
	NO <sub>3</sub> —N	...	0.00415	0.00385	0.00210	0.00186	0.00435	0.00405
	Available—N	0.100	0.09798	0.09844	0.09398	0.09702	0.09771	0.09930
	Total—N	0.100	0.09722	0.09835	0.09497	0.09703	0.09780	0.09928
1 gm surface + 0.1 gm N as NH <sub>4</sub> Cl + 1.0 gm K as KCl.	NH <sub>3</sub> —N	0.100	0.09588	0.09709	0.09510	0.09742	0.09605	0.09690
	NO <sub>3</sub> —N	...	0.00308	0.00265	0.00185	0.00168	0.00395	0.00312
	Available—N	0.100	0.09896	0.09974	0.09695	0.09910	0.10000	0.10002
	Total—N	0.100	0.09900	0.09981	0.09688	0.09901	0.09977	0.09992

TABLE No. 2

Average temperature = 40°C

Unsterile		EXPERIMENTS WITH AMMONIUM SULPHATE AND POTASSIUM CHLORIDE									
Treatment	Original amounts present in grams	Amounts obtained in contact with ZnO surface after 135 days in grams		Amounts obtained in contact with Fe <sub>2</sub> O <sub>3</sub> surface after 150 days in grams		Amounts obtained in contact with Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 180 days in grams		Amounts obtained in contact with BaSO <sub>4</sub> surface after 175 days in grams		Amounts obtained in contact with BaSO <sub>4</sub> surface after 175 days in grams	
		Exposed to light for 582 hrs	Kept in dark	Exposed to light for 661 hrs	Kept in dark	Exposed to light for 768 hrs	Kept in dark	Exposed to light for 736 hrs	Kept in dark	Exposed to light for 736 hrs	Kept in dark
1 gm surface + 0.1 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NH <sub>3</sub> -N	0.02316	0.03316	0.08686	0.09075	0.09159	0.09419	0.09160	0.09376	0.09160	0.09376
	NO <sub>3</sub> -N	0.00271	0.00243	0.00356	0.00342	0.00186	0.00154	0.00168	0.00151	0.00168	0.00151
	Available-N	0.02587	0.03559	0.09042	0.09417	0.09345	0.09573	0.09328	0.09527	0.09328	0.09527
	Total-N	0.02588	0.03564	0.09047	0.09412	0.09347	0.09577	0.09330	0.09526	0.09330	0.09526
1 gm surface + 0.1 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 0.5 gm K as Available-N KCl	NH <sub>3</sub> -N	0.02986	0.03655	0.09286	0.09526	0.09376	0.09647	0.09466	0.09686	0.09466	0.09686
	NO <sub>3</sub> -N	0.00256	0.00229	0.00328	0.00313	0.00146	0.00121	0.00155	0.00137	0.00155	0.00137
	Available-N	0.03242	0.03884	0.09614	0.09839	0.09522	0.09768	0.09621	0.09823	0.09621	0.09823
	Total-N	0.03250	0.03900	0.09618	0.09835	0.09518	0.09770	0.09634	0.09830	0.09634	0.09830
1 gm surface + 0.1 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 1.0 gm K as Available-N KCl	NH <sub>3</sub> -N	0.03645	0.04146	0.09485	0.09705	0.09601	0.09886	0.09789	0.09870	0.09789	0.09870
	NO <sub>3</sub> -N	0.00243	0.00218	0.00306	0.00285	0.00122	0.00090	0.00139	0.00120	0.00139	0.00120
	Available-N	0.03888	0.04364	0.09791	0.09990	0.09723	0.09976	0.09928	0.09990	0.09928	0.09990
	Total-N	0.03896	0.04370	0.09782	0.09995	0.09730	0.09977	0.09925	0.10002	0.09925	0.10002

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TABLE No. 3

Average temperature = 40°C

*Unsterile*

## EXPERIMENTS WITH UREA AND POTASSIUM CHLORIDE

Treatment	Original amounts present in grams	Amounts obtained in contact with				Amounts obtained in contact with			
		ZnO surface after 125 days in grams	Exposed to light for 382 hrs	Kept in dark	grams	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 200 days in grams	Exposed to light for 790 hrs	Kept in dark	grams
1 gm surface + 0.1 gm N as Urea	NH <sub>3</sub> -N	...	0.01416	0.00985	0.00526	0.00456	0.00985	0.00755	0.00938
	NO <sub>3</sub> -N	...	0.01235	0.01025	0.01485	0.01055	0.00651	0.00615	0.00985
	Available-N		0.02651	0.02010	0.02011	0.01511	0.01636	0.01370	0.01923
	Total-N	0.100	0.08297	0.09126	0.08787	0.09337	0.08297	0.08478	0.08467
1 gm surface + 0.1 gm N as Urea + 0.5 gm K as KCl	NH <sub>3</sub> -N	...	0.01186	0.00865	0.00385	0.00345	0.00819	0.00676	0.00786
	NO <sub>3</sub> -N	...	0.01046	0.00915	0.01135	0.00925	0.00575	0.00560	0.00922
	Available-N		0.02232	0.01780	0.01520	0.01270	0.01394	0.01236	0.01708
	Total-N	0.100	0.08667	0.09467	0.09060	0.09656	0.08687	0.08857	0.08817
1 gm surface + 0.1 gm N as Urea + 1.0 gm K as KCl	NH <sub>3</sub> -N	...	0.00895	0.00706	0.00316	0.00285	0.00700	0.00600	0.00686
	NO <sub>3</sub> -N	...	0.00825	0.00775	0.00985	0.00815	0.00511	0.00495	0.00867
	Available-N		0.01720	0.01481	0.01301	0.01100	0.01211	0.01095	0.01553
	Total-N	0.100	0.09067	0.09867	0.09350	0.09968	0.09080	0.09267	0.09178

TABLE No. 4

*Unsterile*

Percentage loss of Nitrogen from Ammonium chloride					Average temperature = 40°C	
Concentration of K added as KCl in grams	With Fe <sub>2</sub> O <sub>3</sub> surface after 75 days		With Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 180 days		With BaSO <sub>4</sub> surface after 145 days	
	Exposed to light for 382 hrs	Kept in dark for 768 hrs	Exposed to light for 768 hrs	Kept in dark for 618 hrs	Exposed to light for 618 hrs	Kept in dark for 618 hrs
--	6.08	4.68	6.80	4.93	4.74	2.90
0.5	2.78	1.65	5.03	2.97	2.20	0.72
1.0	1.00	0.19	3.12	0.99	0.23	0.08

TABLE No. 5

*Unsterile*

Percentage loss of Nitrogen from Ammonium sulphate							Average temperature = 40°C	
Concentration of K added as KCl in grams	With ZnO surface after 135 days		With Fe <sub>2</sub> O <sub>3</sub> surface after 150 days		With Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 180 days		With BaSO <sub>4</sub> surface after 175 days	
	Exposed to light for 582 hrs	Kept in dark for 582 hrs	Exposed to light for 661 hrs	Kept in dark for 661 hrs	Exposed to light for 768 hrs	Kept in dark for 768 hrs	Exposed to light for 736 hrs	Kept in dark for 736 hrs
--	74.12	64.34	9.53	5.88	6.53	4.23	6.70	4.74
0.5	67.50	61.00	3.82	1.65	4.82	2.30	3.66	1.70
1.0	61.04	56.30	2.18	0.05	2.70	0.23	0.75	Nil

TABLE No. 6

*Unsterile*

Percentage loss of Nitrogen from Urea							Average temperature = 40°C	
Concentration of K added as KCl in grams	With ZnO surface after 125 days		With Fe <sub>2</sub> O <sub>3</sub> surface after 165 days		With Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> surface after 200 days		With BaSO <sub>4</sub> surface after 200 days	
	Exposed to light for 382 hrs	Kept in dark for 382 hrs	Exposed to light for 702 hrs	Kept in dark for 702 hrs	Exposed to light for 790 hrs	Kept in dark for 790 hrs	Exposed to light for 79 hrs	Kept in dark for 79 hrs
--	17.03	8.74	12.13	6.63	17.03	15.22	15.33	13.22
0.5	13.33	5.33	9.40	3.44	13.13	11.43	11.83	9.33
1.0	9.33	1.33	6.50	0.32	9.20	7.33	8.22	5.10

*Unsterile*

TABLE No. 7

Percentage of  $\text{NH}_3\text{—N}$  transformed into  $\text{NO}_3\text{—N}$  from Ammonium chloride  
Average temperature =  $40^\circ\text{C}$ .

Concentration of K added as KCl in grams	With $\text{Fe}_2\text{O}_3$ surface after 75 days		With $\text{Ca}_3(\text{PO}_4)_2$ surface after 180 days		With $\text{BaSO}_4$ surface after 145 days	
	Exposed to light for 582 hrs	Kept in dark	Exposed to light for 768 hrs	Kept in dark	Exposed to light for 618 hrs	Kept in dark
—	45.41	51.38	25.43	28.41	50.30	60.34
0.5	59.78	71.17	29.49	38.43	65.51	85.28
1.0	74.77	91.05	37.76	65.12	100	100

*Unsterile*

TABLE No. 8

Percentage of  $\text{NH}_3\text{—N}$  transformed into  $\text{NO}_3\text{—N}$  from Ammonium sulphate  
Average temperature =  $40^\circ\text{C}$ .

Concentration of K added as KCl in grams	With ZnO surface after 135 days		With $\text{Fe}_2\text{O}_3$ surface after 150 days		With $\text{Ca}_3(\text{PO}_4)_2$ sur- face after 180 days		With $\text{BaSO}_4$ surface after 175 days	
	Exposed to light for 582 hrs	Kept in dark	Exposed to light for 661 hrs	Kept in dark	Exposed to light for 768 hrs	Kept in dark	Exposed to light for 736 hrs	Kept in dark
—	3.53	3.63	27.09	36.98	22.12	26.51	20.00	24.19
0.5	3.65	3.61	45.94	66.03	23.40	34.28	29.03	43.63
1.0	3.82	3.72	59.61	96.61	30.58	78.94	65.88	92.32

*Unsterile*

TABLE No. 9

Percentage of  $\text{NO}_3\text{—N}$  present in the available Nitrogen obtained from Urea  
Average temperature =  $40^\circ\text{C}$ .

Concentration of K added as KCl in grams	With ZnO surface after 125 days		With $\text{Fe}_2\text{O}_3$ surface after 165 days		With $\text{Ca}_3(\text{PO}_4)_2$ sur- face after 200 days		With $\text{BaSO}_4$ surface after 200 days	
	Exposed to light for 382 hrs	Kept in dark	Exposed to light for 702 hrs	Kept in dark	Exposed to light for 790 hrs	Kept in dark	Exposed to light for 790 hrs	Kept in dark
—	46.58	50.99	73.84	69.82	39.79	44.89	51.22	53.78
0.5	46.86	51.40	74.67	72.83	41.25	45.31	53.93	56.15
1.0	47.97	52.33	75.71	74.09	42.19	45.21	55.83	57.95

TABLE No. 10

Average temperature=30°C.

EXPERIMENTS WITH AMMONIUM SULPHATE, POTASSIUM CHLORIDE AND CALCIUM CHLORIDE										
Sterile	Treatment	Original amount present in grams	Amounts obtained in contact with				Amounts obtained in contact with			
			ZnO surface after 190 day in grams	Fe <sub>2</sub> O <sub>3</sub> surface after 205 days in grams	MnO <sub>2</sub> surface after 220 days in grams	TiO <sub>2</sub> surface after 235 days in grams				
			Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark
1 gm surface + 0.01 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		NH <sub>3</sub> —N	0.010	0.00234	0.00326	0.00660	0.00711	0.00467	0.00600	0.00750
		NO <sub>3</sub> —N	...	0.00096	0.00085	0.00052	0.00046	0.00058	0.00046	0.00040
		Available—N	0.010	0.00330	0.00411	0.00712	0.00757	0.00525	0.00646	0.00040
		Total—N	0.010	0.00332	0.00412	0.00714	0.00758	0.00527	0.00645	0.00798
1 gm surface + 0.01 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 0.1 gm K as KCl		NH <sub>3</sub> —N	0.010	0.00260	0.00357	0.00700	0.00742	0.00514	0.00650	0.00789
		NO <sub>3</sub> —N	...	0.00093	0.00082	0.00049	0.00043	0.00055	0.00044	0.00042
		Available—N	0.010	0.00353	0.00439	0.00749	0.00785	0.00569	0.00694	0.00831
		Total—N	0.010	0.00354	0.00441	0.00750	0.00787	0.00570	0.00696	0.00832
1 gm surface + 0.01 gm N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 0.1 gm Ca as CaCl <sub>2</sub>		NH <sub>3</sub> —N	0.010	0.00536	0.00720	0.00826	0.00859			
		NO <sub>3</sub> —N	...	0.00086	0.00071	0.00047	0.00041			
		Available—N	0.010	0.00622	0.00791	0.00873	0.00900			
		Total—N	0.010	0.00623	0.00790	0.00872	0.00901			



TABLE No. 11

Average temperature = 30°C

EXPERIMENTS WITH AMMONIUM NITRATE, POTASSIUM CHLORIDE AND CALCIUM CHLORIDE										
<i>Sterile</i>	Treatment	Original amounts present in grams	Amounts obtained in contact with ZnO surface after 190 days in grams		Amounts obtained in contact with Fe <sub>2</sub> O <sub>3</sub> surface after 205 days in grams		Amounts obtained in contact with MnO <sub>2</sub> surface after 220 days in grams		Amounts obtained in contact with TiO <sub>2</sub> surface after 235 days in grams	
			Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark
	1 gm surface + 0.01 gm N as NH <sub>4</sub> NO <sub>3</sub>	NH <sub>3</sub> —N 0.005 NO <sub>3</sub> —N 0.005 Available—N 0.010 Total—N 0.010	0.00200 0.00515 0.00715 0.00716	0.00250 0.00515 0.00765 0.00764	0.00235 0.00515 0.00750 0.00751	0.00341 0.00510 0.00851 0.00853	0.00170 0.00525 0.00695 0.00700	0.00234 0.00521 0.00755 0.00757	0.00300 0.00520 0.00820 0.00823	0.00334 0.00518 0.00852 0.00853
	1 gm surface + 0.01 gm N as NH <sub>4</sub> NO <sub>3</sub> +0.1 gm K as KCl	NH <sub>3</sub> —N 0.005 NO <sub>3</sub> —N 0.005 Available—N 0.010 Total—N 0.010	0.00226 0.00515 0.00741 0.00743	0.00276 0.00515 0.00791 0.00793	0.00268 0.00515 0.00783 0.00785	0.00375 0.00510 0.00885 0.00884	0.00200 0.00525 0.00725 0.00728	0.00250 0.00521 0.00771 0.00775	0.00327 0.00520 0.00847 0.00849	0.00358 0.00518 0.00876 0.00880
	1 gm surface + 0.01 gm N as NH <sub>4</sub> NO <sub>3</sub> +0.1 gm Ca as CaCl <sub>2</sub>	NH <sub>3</sub> —N 0.005 NO <sub>3</sub> —N 0.005 Available—N 0.010 Total—N 0.010	0.00345 0.00511 0.00856 0.00858	0.00389 0.00510 0.00899 0.00905	0.00341 0.00513 0.00854 0.00853	0.00469 0.00504 0.00973 0.00975				

TABLE No. 12

Sterile	EXPERIMENTS WITH UREA AND POTASSIUM CHLORIDE				Average temperature=30°C					
	Treatment	Original amounts present in grams	Amounts obtained in contact with ZnO surface after 240 days in grams	Amounts obtained in contact with Fe <sub>2</sub> O <sub>3</sub> surface after 240 days in grams	Amounts obtained in contact with MnO <sub>2</sub> surface after 240 days in grams	Amounts obtained in contact with TiO <sub>2</sub> surface after 240 days in grams				
			Exposed to light	Kept in dark	Exposed to light	Kept in dark	Exposed to light	Kept in dark		
1 gm surface + 0.01 gms N as Urea		NH <sub>3</sub> —N	0.00050	0.00042	0.00058	0.00050	0.00063	0.00054	0.00039	0.00082
		NO <sub>3</sub> —N	0.00072	0.00058	0.00063	0.00054	0.00067	0.00060	0.00085	0.00075
		Available—N	0.00122	0.00100	0.00121	0.00104	0.00130	0.00114	0.00184	0.00157
		Total—N0.010	0.00750	0.00852	0.00781	0.00893	0.00815	0.00937	0.00832	0.00938
1 gm surface + 0.01 Urea + 0.1 gm K as KCl		NH <sub>3</sub> —N	0.00046	0.00038	0.00054	0.00046	0.00058	0.00050	0.00089	0.00075
		NO <sub>3</sub> —N	0.00067	0.00055	0.00059	0.00050	0.00063	0.00057	0.00078	0.00069
		Available—N	0.00113	0.00093	0.00113	0.00096	0.00121	0.00107	0.00167	0.00144
		Total—N0.010	0.00815	0.00910	0.00852	0.00957	0.00833	0.00937	0.00893	0.00989

TABLE No. 13

Average temperature = 30°C

Sterile

Percentage loss of Nitrogen from Ammonium sulphate

	With ZnO surface after 190 days Exposed to light	With Fe <sub>2</sub> O <sub>3</sub> surface after 205 days Exposed to light	With MnO <sub>2</sub> surface after 220 days Exposed to light	With TiO <sub>2</sub> surface after 235 days Exposed to light
0.1 gm K as KCl	66.80	58.80	24.20	20.20
0.1 gm Ca as Ca Cl <sub>2</sub>	64.60	55.90	21.30	16.80
	37.70	21.00	9.90	...
				15.80
				12.20
				...

TABLE No. 14

Average temperature = 30°C

Sterile

Percentage loss of Nitrogen from Ammonium nitrate

	With ZnO surface after 190 days Exposed to light	With Fe <sub>2</sub> O <sub>3</sub> surface after 205 days Exposed to light	With MnO <sub>2</sub> surface after 220 days Exposed to light	With TiO <sub>2</sub> surface after 235 days Exposed to light
0.1 gm K as KCl	28.40	23.60	14.70	14.70
0.1 gm Ca as Ca Cl <sub>2</sub>	25.70	20.70	11.60	17.70
	14.20	9.50	2.50	15.10
			...	...
				12.00
				...

TABLE No. 15

Average temperature = 30°C

Sterile

Percentage loss of Nitrogen from Urea

	With ZnO surface after 240 days Exposed to light	With Fe <sub>2</sub> O <sub>3</sub> surface after 240 days Exposed to light	With MnO <sub>2</sub> surface after 240 days Exposed to light	With TiO <sub>2</sub> surface after 240 days Exposed to light
0.1 gm K as KCl	25.00	14.80	10.70	14.80
	18.50	9.00	4.30	10.70
				6.30
				2.10
				1.10

Sterile

TABLE No. 16

Percentage of  $\text{NH}_3\text{—N}$  transformed into  $\text{NO}_3\text{—N}$  from Ammonium sulphate  
Average temperature =  $30^\circ\text{C}$

	With ZnO surface after 190 days Exposed to light	With $\text{Fe}_2\text{O}_3$ surface after 205 days Exposed to light	With $\text{MnO}_2$ surface after 220 days Exposed to light	With $\text{TiO}_2$ surface after 235 days Exposed to light
0.1 gm K as KCl	12.53	15.30	10.88	11.50
0.1 gm Ca as $\text{CaCl}_2$	12.57	16.33	11.32	12.57
...	18.54	27.02	...	19.90
		25.36		23.13

Sterile

TABLE No. 17

Percentage of  $\text{NH}_3\text{—N}$  transformed into  $\text{NH}_3\text{—N}$  from Ammonium nitrate  
Average temperature =  $30^\circ\text{C}$

	With ZnO surface after 190 days Exposed to light	With $\text{Fe}_2\text{O}_3$ surface after 205 days Exposed to light	With $\text{MnO}_2$ surface after 220 days Exposed to light	With $\text{TiO}_2$ surface after 235 days Exposed to light
0.1 gm K as KCl	5.00	6.00	7.553	7.89
0.1 gm Ca as $\text{CaCl}_2$	5.474	6.697	8.334	10.00
...	7.097	9.009	...	11.561
				12.676

Sterile

TABLE No. 18

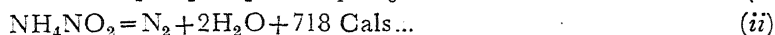
Percentage of  $\text{NO}_3\text{—N}$  present in the available Nitrogen obtained from Urea  
Average temperature =  $30^\circ\text{C}$

	With ZnO surface after 240 days Exposed to light	With $\text{Fe}_2\text{O}_3$ surface after 240 days Exposed to light	With $\text{MnO}_2$ surface after 240 days Exposed to light	With $\text{TiO}_2$ surface after 240 days Exposed to light
0.1 gm K as KCl	59.016	58.00	51.54	46.19
...	59.29	59.14	52.07	47.77
				47.92

All the results with sterile set including those for the nitrogen loss and nitrate formation closely resemble our results obtained with unsterile sets. The nitrogen loss is less marked with ammonium nitrate when compared with ammonium sulphate. This is because the nitrate ion, already present in ammonium nitrate, retards the rate of formation of nitrate ion from ammonium ion and hence there is less loss of nitrogen. The results recorded also show that calcium chloride has a significant effect in checking the nitrogen loss more effectively than potassium chloride and accordingly produces greater percentage of nitrate.

The results from both unsterile and sterile sets of experiments, therefore, lead to the following conclusions :—

1. Where the loss of nitrogen is less marked there is greater nitrate formation and where the loss of nitrogen is greater there is smaller nitrate formation. This is due to the fact that during nitrification of the nitrogenous compounds an unstable intermediate product ammonium nitrite is formed which can undergo both oxidation and decomposition as follows :—



In the former case the reaction number (i) is more prominent than the reaction number (ii) and in the latter case reaction number (ii) is more prominent than the reaction number (i). This also explains why we have obtained less nitrate formation in light than in the dark. It seems that in light reaction number (ii) is more prominent than the reaction number (i).

2. The presence of potassium or calcium chloride in the system increases nitrate formation and greater the concentration of potassium or calcium chloride added in the system, greater is the percentage of nitrate formation from ammonium salts and urea. This may be explained from the viewpoint that when these salts are present in the system they can react with the unstable substance ammonium nitrite formed in the system in the nitrification of ammonium sulphate and urea and generate nitrite of the alkali or alkaline earth metal, which being more stable than ammonium nitrite, remains in the system and can slowly oxidize into nitrates of alkali or alkaline earth metal. Again the presence of either potassium or calcium chloride in solution increases the ionic strength of the medium which is liable to check the decomposition of ammonium nitrite due to secondary salt effect according to the following equation\* :—

$$\log k - \log k_0 = -2.04 \sqrt{\mu}$$

where  $\mu$  is the ionic strength of the added neutral salt,  $k$  is the velocity constant in presence of a neutral salt and  $k_0$  is the velocity constant without the neutral salt. Our results show that calcium solution which obviously provides greater ionic strength checks the loss of nitrogen more effectively than potassium chloride solution of the same concentration. Gopala Rao<sup>5</sup> has also noted that the photosensitized oxidation of ammonia using titanium oxide as surface is retarded by traces of salts of colourless cation like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ba}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Al}^{+++}$  etc., and that greater the valency of cations more effectively they act as retarders.

3. The experimental results obtained by us also show that even under completely sterile conditions there is appreciable nitrification, nitrogen loss and marked influence of potassium or calcium chloride in checking the nitrogen loss and increasing the percentage of nitrate formation when urea or ammonium salts in

solutions are mixed with chemical surfaces and allowed to oxidize. This conclusively proves that nitrification and nitrogen loss are possible even in absence of bacteria both in light as well as in the dark. It appears, therefore, that ammonium salts or urea can undergo slow oxidation on different surfaces with the formation of nitrites and nitrates even in absence of nitrite and nitrate formers.

All these results not only throw a considerable light on the use of mixed fertilizers both in tropical and non-tropical countries but also are of significance in explaining the true mechanism of the formation of nitre beds under natural conditions.

In a previous paper Dhar<sup>20</sup> has advanced a new theory explaining the formation of nitrebeds under natural conditions. The basic idea of this theory is that the source of all nitre deposits is naturally occurring organic substance or excreta of birds or animals. From our experimental results discussed before, it can be inferred that when nitrogen rich compounds like blood, meat meal, fish, hippuric acid, urea, uric acid, guano or other bird or animal excreta are mixed with minerals and allowed to undergo slow oxidation in air and light in presence of every small amounts of sand or soil or silicates, a large amount of nitrogen is lost in the gaseous state along with the formation of sodium, potassium, ammonium, calcium and magnesium nitrates, etc. If the concentration of the minerals present in or added to the system is large there is more nitrate formation. It is well-known, that the following reactions take place in the industrial production of nitric acid from the oxidation of gaseous ammonia in presence of the different catalysts.

- (1)  $4\text{NH}_3 + 5\text{O}_2 = 4\text{NO} + 6\text{H}_2\text{O} + 215.6 \text{ Cal.}$
- (2)  $2\text{NH}_3 + 3\text{O}_2 = 2\text{HNO}_3 + 2\text{H}_2\text{O} + 153.7 \text{ Cal.}$
- (3)  $\text{NH}_3 + 2\text{O}_2 = \text{HNO}_3 + \text{H}_2\text{O} + 80.9 \text{ Cal.}$
- (4)  $4\text{NH}_3 + 3\text{O}_2 = 2\text{N}_2 + 6\text{H}_2\text{O} + 302.0 \text{ Cal.}$
- (5)  $4\text{NH}_3 + 7\text{O}_2 = 4\text{NO}_2 + 6\text{H}_2\text{O} + 269.3 \text{ Cal.}$
- (6)  $4\text{NH}_3 + 6\text{NO} = 5\text{N}_2 + 6\text{H}_2\text{O} + 431.6 \text{ Cal.}$

The main object in nitric acid industry is to convert the ammonia into its oxidised products quickly because when ammonia is present in the mixture of gases containing nitrous fumes it reacts readily with nitrous acid,  $\text{NO}_2$ ,  $\text{NO}_3$ , etc. and liberates gaseous nitrogen. When ammonium salts, urea, uric acid, proteins, oil cakes, etc., are added to soil, the first product formed is ammonia which in its turn is converted slowly into oxidized products like nitrites and nitrates. It is clear, therefore, that under ordinary soil conditions the whole of the ammonia present in the system or produced from proteins urea, uric acid, etc., cannot be readily converted into its oxidized products and hence ammonium ion and occasionally free ammonia have to co-exist with nitrite ion or nitrous acid, nitrate ion or nitric acid and hence marked decomposition takes place with evolution of gaseous nitrogen. If, however, this decomposition takes place in presence of some mineral matters the loss of nitrogen is checked and there is greater nitrate formation. The evolution of nitrogen from nitrogenous compounds by this process has to be more pronounced than in the catalytic oxidation by platinum as in the industrial process, because the oxidation of ammonia at high temperature has a much larger velocity than at the ordinary temperature in soils or in presence of pure chemical surfaces. It is quite clear, therefore, that the mechanism of the oxidation of ammonia, ammonium salts, urea, proteins, etc., is more or less identical

as in industrial operations, only in soils at the ordinary temperature the formation of gaseous nitrogen is more marked than in the industrial process.

Again from a numerous publications<sup>6,7</sup> from this laboratory we have clearly shown that when organic matter, rich in carbon, undergoes oxidation in presence of a surface, there is appreciable fixation of nitrogen. The fixed nitrogen consists of proteins and perhaps some amino acids and small quantities of ammonium salts. If, in this system, sodium, potassium or calcium salts or carbonates, bicarbonates or phosphates are added, the loss of nitrogen during the process of nitrification is appreciably retarded as the decomposition of the unstable substance, ammonium nitrite, formed as an intermediate product during nitrification of the nitrogenous compounds, is considerably checked in presence of these substances, due to the formation of stable nitrites of these salts which in their turn are oxidized to nitrates. It is clear, therefore, that all kinds of carbonaceous substances and nitrogen rich compounds in course of time can be completely converted into nitrates of alkali or alkaline earths because the former group of substances are unstable in presence of air and can undergo slow oxidation to carbonic acid, sulphuric acid, ammonia, nitrite and nitrate. In this process, if there is any iodide present under certain conditions, iodates are formed. Iodates may react with chlorides to form chlorates and perchlorates which also occur in nitre deposits in small quantities. Recently we<sup>8</sup> have shown that phosphates are able to intensify nitrogen fixation in soils or chemical surfaces. Hence it seems that sea weeds rich in potassium compounds with a C/N ratio greater than 10 can with difficulty form nitre beds as has been postulated by many workers. But under certain conditions sea weeds impregnated with guano which is rich in phosphate along with fish or animal body or excreta of birds or animals, undergoing slow oxidation in air, can fix large quantities of nitrogen. This fixed nitrogen and the nitrogenous compounds present in the system can undergo nitrification in air and can pass into mineral nitrates. This is a probable explanation of the formation of nitre beds from sea weed or other organic substances as are found in Chile, Peru and in islands which are inhabited by sea birds. Animal urine or dung mixed with plant-ash can slowly undergo oxidation and can form nitrates in the end. In course of time the whole of the organic matter is likely to be oxidized under aerobic condition and can pass into mineral nitrates. In this process bacteria, sunlight or artificial light, soil or sand and some solid surfaces of pure inorganic oxides are helpful in nitrification. It seems, therefore, that in the formation of nitre beds it is necessary to have salts of alkali or alkaline earth metals or other minerals in presence of small quantity of alkali, because the presence of these salts and alkalinity checks the decomposition of ammonium nitrite. Thus, the loss of nitrogen during nitrification of all the carbonaceous and nitrogenous compounds is decreased and fuller conversion to nitrate becomes possible. Hence the true formation of nitre deposits of the World, where the amount of nitrate present is large, is due possibly to the photo-chemical, surface and bacterial nitrification under aerobic condition of all kinds of organic materials and nitrogen rich compounds in presence of little alkali, phosphates and mineral matters soluble in water.

Goldschmidt<sup>9</sup> has discarded the theory of nitre beds formation from *kelp* and other sea weeds as he believes that this hypothesis is contradicted by every kind of geological evidence. Similarly he is also against the view that the guano and excreta of birds is the source of nitre beds. He has considered that nitrates may originate from magmatic rocks or sediments or from electrical discharge in air. It is well-known that rain water contains 2 to 14 lbs or more of

nitrate per acre but it is difficult to assume that such a dilute nitrate solution can be the source of nitre beds. It also seems doubtful that nitrate in nitre beds have originated from volcanic rocks or by the oxidative weathering of ordinary sediments. The experimental support seems to rest on the viewpoint that organic matter in the process of its decomposition specially in presence of phosphates and mineral matters soluble in water and a suitable surface form the true source of nitre deposits of the world. It is well-known that in all countries potassium nitrate was manufactured from nitre beds rich in proteins, urea, uric acid along with carbonaceous compounds and ashes. The evidence in favour of the view that nitrification and nitre bed formation in natural conditions is more a chemical and surface oxidation reaction than bacterial process are as follows :—

(1) Results obtained by us show that nitrification and nitrate formation can take place in completely sterile condition in contact with various solid surfaces where no bacterial action is possible.

(2) Omeliansky<sup>10</sup> and Meyerhof<sup>11</sup> have noted that for bacterial action high concentration of ammonia or nitrite is harmful; over 0.3% interferes with the process. In certain localities, the so called "nitre spots" a high concentration of nitrite and nitrate as much as 5% or more of the bulk has been formed. The biological explanation is insufficient to explain the accumulation of nitrites and nitrates in such high concentration in nitre spots.

(3) Bazarewsky<sup>12</sup>, Koch<sup>13</sup> and Kelley<sup>14</sup> and co-workers have found that the nitrification process is most active near the surface of the soil. According to Prescott and Piper<sup>15</sup> nearly 80% of the nitrate accumulation takes place in the first 3½ inches of the soil from the surface. In tropical countries during summer the soil temperature is not much below 50° even at the depth of 3½ inches from the surface, although, for bacterial nitrification the optimum temperature is 35°C. In cold countries in summer the soil temperature may rise to 35°C or more, the optimum temperature for bacterial nitrification there is stated to be 25°C. A periodic variation of nitrite and nitrate content of soils has been noticed by several workers<sup>16</sup>, and their presence in soil rises to a maximum in summer and falls off to a minimum in winter.

In certain South African soil, nitrification is attributed largely to photo-chemical oxidation on the soil surface, following adsorption of atmospheric ammonia on soil colloids.<sup>17</sup> Puri<sup>18</sup> has also stated that nitrites continually oxidize to nitrates in soil independent of microbiological phenomenon. This seems to be the reason why in nature nitrates occur in abundance and nitrites in only small amounts. According to Rossi,<sup>19</sup> when rain or irrigation leaches nitrites and nitrates from superficial soil layers in contact with air, rapid nitrification occurs, particularly intense at relatively high temperatures, which appears to be purely physico-chemical phenomenon independent of micro-organic activity. Physical chemists, therefore, consider that the vast deposits of nitrate occurring naturally in the earth as saltpetre and other compounds were formed in physico-chemical instead of biological reactions. It is gratifying to note that Sir John Russell is in general agreement with our views as is evident from his following lines : "The Chilean deposits could hardly have been formed by the ordinary nitrification process, for this involves the oxidation of ammonia to ammonium nitrite which readily loses nitrogen. If, however, the oxidation took place in presence of sodium and potassium salts their nitrites being more stable would persist and be further oxidized to nitrate which could accumulate. These conditions would be satisfied if the original material had been seaweed."



## SUMMARY

Nitrogen rich compounds can undergo nitrification in contact with solid surfaces of some inorganic materials both under sterile and unsterile conditions in light as well as in the dark and this process is associated with nitrogen loss and nitrate formation. The loss of nitrogen is due to the formation and decomposition of the unstable substance ammonium nitrite which readily breaks up into water and nitrogen gas.

In every case the presence of potassium and calcium chloride in the system retards the loss of nitrogen and increase nitrate formation and greater the concentration of potassium or calcium chloride added smaller is the loss of nitrogen and greater is the nitrate formation. This is due to the fact that both potassium and calcium salts form stabler nitrites of these metals than ammonium nitrite and can slowly oxidize into nitrate of these metals. Again the presence of either potassium or calcium chloride in solution increases the ionic strength of the medium and retard the decomposition of ammonium nitrite due to secondary salt effect.

The formation of nitre beds in nature has been explained possibly due to the photo-chemical, surface and bacterial nitrification, under aerobic condition, of all kinds of organic materials and nitrogen rich compounds in presence of little alkali, phosphates and mineral matters soluble in water.

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FIXATION OF NITROGEN IN ALKALI AND ORDINARY SOILS  
BY ADDING LIGNITE OR A MIXTURE OF  
LIGNITE AND STRAW

By N. R. DHAR and T. N. CHOJER

*Sheila Dhar Institute of Soil Science, University of Allahabad.*

INTRODUCTION

In previous communications from this laboratory, Dhar and co-workers<sup>1-3</sup> have shown that when carbonaceous substances like molasses, straw, glucose, cane sugar, glycerol, starch, saw-dust, etc., etc., are mixed with soil, sand or other metallic oxides like  $\text{Fe}_2\text{O}_3$ ,  $\text{MnO}_2$ ,  $\text{ZnO}$  etc., and allowed to undergo slow oxidation in air the nitrogen of the system increases.

The purpose of this paper is to study the nitrogen fixation in very bad alkali soil of pH 10.4 and an ordinary soil by the addition of lignite (obtained from Palana, Madras Presidency) alone and a mixture of lignite and wheat straw.

The effect of addition of phosphate and certain trace elements like molybdenum and vanadium on nitrogen fixation has also been studied.

EXPERIMENTAL PROCEDURE

200 gms of soil after being passed through a sieve of 50 mesh per inch was taken in white enamel dishes. To this soil was added 0.5% of carbon as lignite. In some cases 0.25% of carbon as lignite and 0.25% of carbon as straw was added. In certain other cases 0.1% of  $\text{P}_2\text{O}_5$  as  $\text{Na}_2\text{HPO}_4$  and 0.005% of molybdenum and vanadium as potassium molybdate and vanadium pentaoxide were added to the system. The contents of the plates were thoroughly mixed in an agate pestle and mortar. These dishes were exposed in the light of a 500 watts electric bulb hung over the table at a distance of two feet. A similar set of dishes containing the same amount of materials was placed beside these dishes covered with a thick black cloth to exclude light completely. The contents of the dishes were stirred on alternate day to facilitate the oxidation of the organic matter. 7—8% of distilled water was added to the exposed dishes daily and on every fourth day to the covered ones. From time to time a portion was sampled out and analysed for total carbon and total nitrogen.

The total carbon was estimated by Robinson, Mclean and Williams<sup>4</sup> method while the nitrogen was determined by salicylic acid reduction method.<sup>5</sup>

Period of Exposure	Total Carbon	Total Nitrogen	Oxidation percentage of carbon	Efficiency (amount of nitrogen fixed m. gms per gram carbon oxidized)
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200 gms of alkali soil + 0.25 % of C as lignite + 0.25% of C as Straw

*Exposed*

0 days	0.613	0.03955	—	—
120 „	0.4342	0.04167	29.1	11.3
180 „	0.3487	0.04248	43.1	11.1

*Covered*

0 days	0.613	0.03955	—	—
120 „	0.4743	0.04042	22.6	6.3
180 „	0.4146	0.04076	32.3	6.1

200 gms of alkali soil + 0.25% of C as lignite + 0.25% of C as straw + 0.005% of  $V_2O_5$

*Exposed*

0 days	0.6125	0.03953	—	—
120 „	0.4212	0.04213	31.1	13.4
180 „	0.3351	0.04319	45.3	13.1

*Covered*

0 days	0.613	0.03955	—	—
120 „	0.4609	0.04075	24.8	7.9
180 „	0.4018	0.04317	34.4	7.7

200 gms of alkali soil + 0.25% of C as lignite + 0.25% of C as straw + 0.005% of Molybdenum

*Exposed*

0 days	0.6132	0.03953	—	—
120 „	0.4165	0.04237	32.05	14.4
180 „	0.3302	0.04356	46.1	14.2

*Covered*

0 days	0.613	0.03953	—	—
120 „	0.4534	0.04094	26.1	8.7
180 „	0.3968	0.04138	35.3	8.5

Period of Exposure	Total Carbon	Total Nitrogen	Oxidation percentage of carbon	Efficiency (amount of nitrogen fixed in m. gms. per gram of Carbon oxidized)
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200 gms of alkali soil + 0.25% of C as lignite + 0.25% of C as straw + 0.1%  $P_2O_5$

*Exposed*

0 days	0.613	0.03955	—	—
120 „	0.4088	0.04242	33.5	16.5
180 „	0.3235	0.04424	47.4	16.2

*Covered*

0 days	0.612	0.03955	—	—
120 „	0.4465	0.04121	27.2	9.9
180 „	0.3925	0.04166	37.2	9.6

200 gms ordinary soil + 0.25% of C as lignite + 0.25% of C as straw

*Exposed*

0 days	0.736	0.04011	—	—
120 „	0.5587	0.04317	24.1	17.3
180 „	0.4851	0.04424	34.1	16.9

*Covered*

0 days	0.7356	0.04011	—	—
120 „	0.6028	0.04133	18.1	9.2
180 „	0.5638	0.04164	23.4	8.9

200 gms of ordinary soil + 0.25% of C as lignite + 0.25% of Carbon as straw + 0.005% of  $V_2O_5$

*Exposed*

0 days	0.7362	0.04015	—	—
120 „	0.5410	0.04429	26.5	26.6
180 „	0.4680	0.04713	36.3	26.3

*Covered*

0 days	0.736	0.04011	—	—
120 „	0.5884	0.04189	20.05	12.1
180 „	0.5472	0.04235	25.6	11.9

Period of Exposure	Total Carbon	Total Nitrogen	Oxidation percentage of carbon	Efficiency (amount of nitrogen fixed in m. gms per gram of Carbon oxidized)
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200 gms ordinary soil + 0.25% of carbon as lignite + 0.25% of Carbon as straw + 0.005% of Mo

*Exposed*

0 days	0.7356	0.04011	—	—
120 „	0.5362	0.04548	27.2	27.4
180 „	0.4608	0.04758	37.4	27.2

*Covered*

0 days	0.736	0.04011	—	—
120 „	0.5836	0.04213	20.0	13.3
180 „	0.5404	0.04267	26.5	13.1

200 gms of ordinary soil + 0.25% of carbon as lignite + 0.25% of Carbon as straw + 0.1%  $P_2O_5$

*Exposed*

0 days	0.7356	0.04014	—	—
120 „	0.5272	0.04610	28.3	28.9
180 „	0.4483	0.04833	39.1	28.6

*Covered*

0 days	0.7356	0.04011	—	—
120 „	0.5740	0.04262	22.1	15.5
180 „	0.5316	0.04319	27.8	15.1

200 gms of alkali soil + 0.5% of Carbon as lignite

*Exposed*

0 days	0.613	0.02968	—	—
150 „	0.5192	0.04053	15.3	9.1

*Covered*

0 days	0.613	0.03965	—	—
150 „	0.5332	0.04008	13.01	5.1

200 gms of alkali soil + 0.4% of Carbon as lignite + 0.005% of  $V_2O_5$

*Exposed*

0 days	0.6127	0.03968	—	—
150 „	0.5132	0.04096	16.2	12.8

*Covered*

0 days	0.613	0.03968	—	—
150 „	0.5291	0.04023	13.7	68

Period of Exposure	Total Carbon	Total Nitrogen	Oxidation percentage of Carbon	Efficiency (amount of nitrogen fixed in m. gms. per gram of Carbon oxidized)
200 gms of alkali soil + 0.5% of Carbon as lignite + 0.006% of Mo				
<i>Exposed</i>				
0 days	0.6127	0.03965	—	—
150 „	0.5102	0.04103	16.7	13.2
<i>Covered</i>				
0 days	0.6127	0.03968	—	—
150 „	0.5211	0.04034	14.9	7.2
200 gms of alkali soil + 0.5% of Carbon as lignite + 0.1% of P <sub>2</sub> O <sub>5</sub>				
<i>Exposed</i>				
0 days	0.613	0.03968	—	—
150 „	0.5012	0.04128	18.2	14.4
<i>Covered</i>				
0 days	0.6127	0.03970	—	—
150 „	0.5130	0.04049	14.9	7.2
200 gms of ordinary soil + 0.1% of Carbon as lignite				
<i>Exposed</i>				
0 days	0.7356	0.04003	—	—
150 „	0.6321	0.04146	14.1	15.2
<i>Covered</i>				
0 days	0.736	0.04003	—	—
150 „	0.6433	0.04089	12.5	9.1
200 gms of ordinary soil + 0.5% of Carbon as lignite + 0.005% of V <sub>2</sub> O <sub>5</sub>				
<i>Exposed</i>				
0 days	0.7362	0.04003	—	—
150 „	0.6254	0.04263	15.2	23.4
<i>Covered</i>				
0 days	0.7362	0.04003	—	—
150 „	0.6381	0.04124	13.3	12.3
200 gms of ordinary soil + 0.5% of Carbon as lignite + 0.005% of Mo				
<i>Exposed</i>				
0 days	0.7358	0.4003	—	—
150 „	0.6204	0.4283	15.7	24.3
<i>Covered</i>				
0 days	0.736	0.04002	—	—
150 „	0.6342	0.04141	13.8	13.4

Period of Exposure	Total Carbon	Total Nitrogen	Oxidation percentage of carbon	Efficiency (amount of nitrogen fixed in m. gms per gram of Carbon oxidized)
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200 gms of ordinary soil + 0.5% of Carbon as lignite + 0.1%  $P_2O_5$

		<i>Exposed</i>		
0 days	0.7362	0.04003	—	—
150 „	0.6125	0.04353	16.8	28.3
		<i>Covered</i>		
0 days	0.736	0.04003	—	—
150 „	0.6275	0.04182	14.7	16.4

#### DISCUSSION

A close study of the above results indicates that when lignite is added to the soil, the nitrogen content of the soil increases. It has also been observed that the oxidation of carbon is greater in the alkali soil than in ordinary soil. This is due to the higher pH *i.e.*, more alkalinity of the alkali soil. But the fixation of nitrogen is greater in case of ordinary soil.

When we compare the results obtained by the addition of lignite alone and a mixture of lignite and straw, it is evident that the mixture of straw and lignite fixes more nitrogen than lignite alone. The oxidation is also greater in case of the mixture.

When we add phosphate, the oxidation increases, more in the alkali soil than in the ordinary soil. The efficiency *i.e.*, the nitrogen fixed in m. gms per gram of carbon oxidized increases very much by the addition of phosphate. The efficiency is greater in the case of ordinary soil than in alkali soil.

The effect of trace elements is also marked. The addition of molybdenum and vanadium increases the efficiency of nitrogen fixation appreciably. It also affects the oxidation of carbon of the system. The oxidation of carbon increases in the order vanadium, molybdenum and phosphate. The nitrogen fixation is also affected in the same order. It seems, therefore that trace elements play a vital role in the fixation of nitrogen.

The light absorbed markedly increases the oxidation of carbon as well as efficiency of nitrogen fixation. It is quite clear from the above results that the fixation of nitrogen is much greater in the sets exposed to light than in the covered ones. This observation is common to all the cases.

In the light of the above observations we can say that the mixture of lignite and straw is better for nitrogen fixation than lignite alone. Phosphate, molybdenum and vanadium are also beneficial and so may be used for the betterment of the Indian soils deficient in nitrogen.

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# INFLUENCE OF PHOSPHATES ON AMINO ACID FORMATION BY THE ACTION OF NITRATES ON CARBOHYDRATES

By N. R. DHAR and T. B. GUHA ROY

*Department of Agricultural Chemistry, Sheila Dhar Institute of Soil Science,  
University of Allahabad*

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The photosynthesis of amino acids have been studied in detail by Dhar and Mukherji.<sup>1</sup> Solutions of glucose and nitrates in presence of different photosensitizers were exposed to artificial light or sunlight and the amino acids were detected by the method of Mitchell and Hamilton.<sup>2</sup> The estimation of the amino acids were made by a Duboscq colorimeter according to the method of Harding and Mclean.<sup>3</sup> They found that the yield is maximum with ammonium nitrate and minimum with sodium nitrate. With potassium nitrate, the yield was intermediate between ammonium and sodium nitrate. If in place of these nitrates, other ammonium salts like ammonium hydroxide or ammonium chloride or ammonium sulphate is taken, no amino acid is photosynthesized. In addition to other facts, they have partly been able to isolate glycine and arginine. The isolation of arginine was confirmed by Nathan<sup>4</sup> by picrate and chloroplatinate methods. We have tried to investigate the influence of various phosphates in the photosynthesis of amino acids.

## EXPERIMENTAL

In three 250 c.c. beakers, 5 c.c's of normal solutions of nitrates of ammonium, sodium and potassium together with 0.25 gms of glucose and 0.05 gms of titania were taken. In separate beakers, the same substances were taken and in each of them were added 0.1 gm of dicalcium phosphate. Similarly solutions were exposed with monocalcium phosphate, tricalcium phosphate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate and tripotassium phosphate, care being taken in each case that the  $P_2O_5$  content remains the same. A beaker containing distilled water was also exposed. The beakers were exposed to 1000 watt bulb and the contents of the beaker were shaken from time to time. After two hours exposure, the solutions were filtered and the filtrate was tested for amino acids by circular paper chromatography of Giri and Rao<sup>5</sup> using butanol-acetic acid and water as solvent and 0.1 per cent ninhydrin in acetone as the colour producing reagent. The amount of solution spotted at the centre of Whatman No. 1 filter paper was exactly 0.05 c.c. in each case. The experimental results are as follows .—



TABLE 1

*Sodium nitrate + Glucose + Titania*

<i>Phosphate added</i>	<i>Amount of amino acid obtained</i>
Monocalcium phosphate	Amount less than without phosphate
Dicalcium phosphate	Amount greater than without phosphate
Tricalcium phosphate	Amount less than without phosphate
Potassium dihydrogen phosphate.	Amount much greater than without phosphate
Dipotassium hydrogen phosphate.	Amount very much greater than without phosphate.
Tripotassium phosphate	Amount greater than without phosphate

It is clear from the foregoing table that the amino acid formation is always better with mono, di and tripotassium phosphates. It is also better with dicalcium phosphate.

TABLE 2

*Potassium nitrate + Glucose + Titania*

<i>Phosphate added</i>	<i>Amount of amino acid obtained</i>
Monocalcium phosphate	Amount less than without phosphate
Dicalcium phosphate	Amount much greater than without phosphate
Tricalcium phosphate	Amount greater than without phosphate
Potassium dihydrogen phosphate.	Amount almost the same as without phosphate
Dipotassium hydrogen phosphate	Amount very much greater than without phosphate
Tripotassium phosphate	Amount much greater than without phosphate

It is thus seen that the formation of amino acids are facilitated by the potassium phosphates and also by dicalcium and tricalcium phosphate.

TABLE 3

*Ammonium nitrate + Glucose + Titania*

<i>Phosphate added</i>	<i>Amount of amino acid obtained</i>
Monocalcium phosphate	Amount less than without phosphate
Tricalcium phosphate	Amount greater than without phosphate
Potassium dihydrogen phosphate.	Amount greater than without phosphate.
Dipotassium hydrogen phosphate.	Amount very much greater than without phosphate.
Tripotassium phosphate	Amount greater than without phosphate

Thus we see, that with the potassium phosphates, the formation of amino acids is greater than without any phosphate. We have also obtained chromatographically that the yield of amino acid is greater with ammonium nitrate and least with sodium nitrate, which is in conformity with Dhar and Mukherji's results. Further the fact that no amino acid is formed with salts of ammonium other than nitrates has been confirmed by our experiments.

Thus we see that with the addition of phosphates, the amount of amino acid formed is greater than without any phosphate. It seems therefore, that phosphates can stabilize amino acids and proteins.

An attempt was made to obtain a quantitative idea of the amounts of amino acids in the above experiments. Several dilutions of L—aspartic acid were prepared and then it was estimated by comparison.

Polson<sup>6,7</sup> described a semiquantitative method of estimating the amino acids. He prepared amino acids of a series of known dilutions and compared the intensity of the spots obtained with ninhydrin.

In our experiments, 3 factors were kept constant.

1. The same amount of liquid was taken at the centre, *i.e.*, 0.05 c.c. in each case.
2. The loss of the introduced amino acid was avoided by making two perpendicular scratches at the centre instead of a round hole for inserting the wick.
3. The amount of ninhydrin solution added was the same in all cases.

So far experiments have been done with ammonium nitrate and the three potassium phosphates. Experiments with calcium phosphates and other nitrates are in progress. The following results have been obtained :

TABLE 4

Amounts of amino acids formed with normal Ammonium nitrate  
after 2 hours exposure.

Without phosphate	0.00056 M
With potassium dihydrogen phosphate	0.00115 M
With dipotassium hydrogen phosphate	0.00145 M
With tripotassium phosphate	0.00142 M

It, therefore, seems that the amount of amino acid formed is maximum with dipotassium hydrogen phosphate and minimum in the absence of phosphate.

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# CHROMATOGRAPHIC DETECTION OF AMINO ACIDS IN SOILS

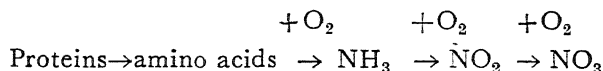
By N. R. DHAR and TIMIR BARAN GUHA ROY

*Department of Agricultural Chemistry, Sheila Dhar Institute of Soil Science,  
University of Allahabad*

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The most important aspect of agricultural chemistry is the nitrogen metabolism in soil and the same is true in the case of amino acid content of soil. Nitrogen in soil humus is present mostly in the form of proteins which is of plant, animal and microbial origin. Proteins are present in about one-third of the total humus in ordinary garden and field soils.<sup>1</sup>

It has been shown by Dhar and co-workers,<sup>2</sup> that when proteins in soils under go nitrification by bacteria, following changes take place :—



It is quite clear therefore, that during protein synthesis in soils, amino acids are formed. Proteins with lignin forms humus like complexes in soils which liberates amino acids on treatment with acids and alkalies. Amino acids like leucine, alanine, aspartic acid, isoleucine, histidine, arginine, and lysine have been detected in soils by several workers.<sup>3,4,5,6</sup> A quantitative method using microbiological method was made by Kojima<sup>7</sup> for estimating aspartic acid, glutamic acid, hydroxyproline, isoleucine, leucine and valine.

Though the amino acids are present in soils in very small quantity, but the introduction of paper chromatography by Consden<sup>8</sup> et al has made the detections of amino acids in soils much easier. Bremner<sup>9</sup>, Davidson, Sowden and Atkinson<sup>10</sup> applied paper chromatography to the identification and quantitative estimation of amino acids in soil organic matter. Biswas and Das<sup>11</sup> used 6N hydrochloric acid for hydrolysis of the soils for the detection of amino acids. Recently, however, we have obtained amino acids by triturating different soil samples with distilled water at the room temperature and filtering it. In the filtrate, amino acids was readily detected by circular paper chromatography developed Giri and Rao.<sup>12</sup>

## EXPERIMENTAL

4 gms of soil along with 8 c. c. of distilled water were taken in a glass pestle and mortar and this was triturated for 10 to 15 minutes. The soil solution was divided into two parts ; one part was filtered and the other was boiled for one minute and then filtered. This was done to see whether the formation of amino acid is more in cold or in hot condition. The filtrate obtained is spotted at the centre of

Whatman No. 1 filter paper, amount in each case being 0.05 c. c. The developing solvent used was butanol—acetic acid-water and 0.1% ninhydrin in acetone as colour reagent. The filter papers were incubated for 5 minutes at 50°C for at this temperature and time, it has been found that the colouration obtained with distilled water is practically nil.

Our experiments with garden soil, good soil, normal soil, bad soil, Swedish soil, compost soils from different composts, fresh soils from many places swrrounding our Institute, etc., show invariably that the formation of amino acids is more in the soils than in distilled water.

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## STUDIES IN PHOSPHATES

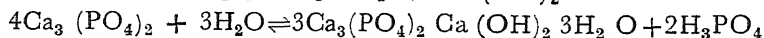
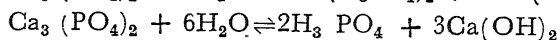
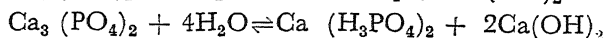
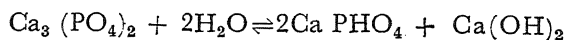
By N. R. DHAR and DHARMENDRA SHARMA

*Sheila Dhar Institute of Soil Science, University of Allahabad*

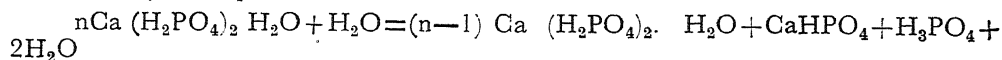
(Received on 14th October, 1954)

It is well-known that phosphates play an important role in maintaining soil fertility and that they are one of the essential ingredients of plant and animal life. In cold country soils the available phosphate status is low as compared to the tropical soils due to the formation of insoluble phosphates of iron and aluminium, etc. Moreover the loss of phosphates by leaching in areas of heavy rainfall has been noticed. Robinson and Jones<sup>1</sup> observed the washing of phosphates in the wet soils of Wales. Hall<sup>2</sup> observed the same in the soils of Rothamsted. Iverson<sup>3</sup> reported loss of phosphate by washing even in Denmark soils where rainfall is moderate. It appears therefore that soil phosphates undergo a series of physico-chemical changes. The manner in which the different soil phosphates are formed and decomposed still remains a controversial point. It is, however, interesting to note here that tricalcium phosphate and ferric phosphate corresponding in composition to the formulae<sup>4</sup>  $\text{Ca}_3 \text{P}(\text{O}_4)_2$  and  $\text{FePO}_4$  are difficult to prepare in the wet way.

It has been observed<sup>5</sup> that when tricalcium phosphate is boiled with water, free phosphoric acid is liberated which can be tested by ammonium molybdate reagent. Further it has been reported that in presence of water the calcium phosphates are unstable and hydrolyse into phosphoric acid and substances containing more lime. The tri salt is supposed to decompose as :

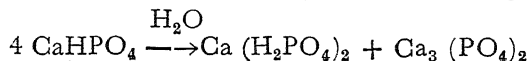


Spring showed that under pressure the monocalcium phosphate decomposes into dicalcium phosphate and phosphoric acid. Erlenmeyer<sup>7</sup> found that the solution of monocalcium phosphate contained more phosphoric acid than corresponded to the original salt. Stoklasa<sup>8</sup> represented the action of water upon the mono salt by the equation :



Joly<sup>9</sup> also made some experiments with this salt. Stoklasa<sup>10</sup> says that the wide variations in the earlier results are due to contaminated results.

Dellatre<sup>11</sup>, Millot<sup>12</sup>, Joly and Soral<sup>13</sup>, Viard<sup>14</sup> observed that when the dicalcium phosphate is boiled with water monocalcium phosphate goes into solution and tricalcium phosphate is precipitated according to



We are of the opinion that as tricalcium phosphate is very unstable in presence of water and is immediately decomposed into a solid richer in lime, the observation of the above workers that tricalcium phosphate is precipitated from dicalcium phosphate does not seem correct. Moreover, monocalcium phosphate does not remain as such in solution because it is converted into a solution richer in phosphoric acid and a solid mainly consisting of dicalcium phosphates. However, there is a great probability of the formation of a solid solution corresponding to  $\text{Ca}_3(\text{PO}_4)_2$ .

Thus the question arises whether the more insoluble phosphates of aluminium and iron also undergo similar changes. It seems probable that these phosphates are also hydrolysed into phosphoric acid and their hydroxides and the amount of hydrolysis should be larger as aluminium hydroxide and ferric hydroxide are weaker bases than calcium hydroxide. Lachowicz<sup>15</sup> has investigated the action of water upon ferric phosphate and he is of the opinion that with continuous leaching of the phosphate by water all the phosphoric acid may be leached out leaving only ferric hydroxide. The maximum period for which the salt was in contact with water was 43 days. With aluminium phosphate Cameron and Hurst<sup>16</sup> have observed that considerable amount of phosphoric acid is washed out. Cameron and Bell<sup>17</sup> found the behaviour of magnesium phosphates to be quite similar to the calcium phosphates.

The present investigation deals with the conductivity and pH changes which the different soil phosphate solutions in water have suffered at 30°C and at 50°C in 6 months. Saturated solutions of ferric phosphate, aluminium phosphate, tricalcium phosphate, dicalcium phosphate, dimagnesium phosphate were made by shaking 1.5 gms of the salt in 100 c.c. of water. The monocalcium phosphate was taken in three concentrations of M/10, M/100 and M/1000, and the electrical conductivity and pH measured with lapse of time. The pH measurements were made by Beckman pH meter and it was found that a considerable amount of hydrolysis took place. This is also obvious from our experiments by mixing equivalent amounts of lime and phosphoric acid when we observed that neutralizations were incomplete due to immediate copious hydrolysis. The following results have been obtained :—

Phosphate	Temp. 30°C				
	Original conductivity after 15 mts	Final conductivity after 6 months	% increase in conductivity	Original pH	Final pH
Tricalcium phosphate	0.000128	0.000505	316.6	5.9	5.1
Dicalcium phosphate	0.000150	0.000180	20.0	9.3	9.4
M/10 Monocalcium phosphate	0.00846	0.0090	7.7	3.3	3.3
M/100 Monocalcium phosphate	0.00134	0.00141	6.0	4.5	4.5
M/1000 Monocalcium phosphate	0.00018	0.00019	5.5	5.4	5.4
Aluminium phosphate	0.00024	0.00074	208.3	8.1	7.2
Ferric phosphate	0.00010	0.00024	140.0	7.2	6.5
Dimagnesium phosphate	0.00049	0.000628	27.2	8.1	7.3

Temp. 50°C

Phosphate	Original conductivity after 15 Mts	Final conductivity after 6 months	% increase in conductivity	Original pH	Final pH
Tricalcium phosphate	0.00024	0.00102	325.0	5.7	4.8
Dicalcium phosphate	0.00036	0.000504	40.0	9.55	6.2
M/10 Monocalcium phosphate	0.01080	0.01090	0.92	3.4	3.4
M/100 Monocalcium Phosphate	0.00173	0.00180	4.0	4.5	4.5
M/1000 Monocalcium phosphate	0.00022	0.000252	13.6	5.35	5.3
Aluminium phosphate	0.00044	0.000128	191.0	7.6	7.0
Ferric phosphate	0.00016	0.000624	289.9	7.35	6.0
Dimagnesium Phosphate	0.000672	0.00139	107.4	7.45	6.8

The above results show that when sparingly soluble phosphates are vigorously shaken with water then in every case the electrical conductivity increases and the pH decreases with time. Two processes take place in the system. Firstly the sparingly soluble salt slowly passes into solution and this produces greater conductivity of the system. There has been a lot of misconception regarding the solubility of lime phosphates in water. If the substances went as such into the solution, then the ratio of lime to  $P_2O_5$  should be same, but apart from this the salt is decomposing and giving more  $P_2O_5$  than lime *e.g.*, the substance in solution always contains more  $P_2O_5$  than lime. Hence by the solubility of these phosphates is meant the total amount of  $P_2O_5$  and lime that has gone into solution. It appears therefore that the dissolved matter as well as the undissolved matter undergoes hydrolysis and in this process also the electrical conductivity increases and the pH decreases. The adsorbed hydrogen ions and phosphate ions on the surface of the solid are slowly given out and in this third process also there may be a feeble increase in conductivity and fall of pH. We are firmly of the opinion that one of the main reasons of increase of conductivity and fall of pH even after such a long period is due to ageing of the solid, when the solid surface loses its reactivity to hold the ions any longer with the same potentiality as before.

The conductivity of the tricalcium phosphate solution increased 316% at 30°C in 6 months, while with aluminium phosphate and ferric phosphate it increased only 208.3% and 140%. The fall of pH in the three salts is nearly same. At 50°C the hydrolysis is intensified and the conductivity of tricalcium phosphate solution increased 325% in the same period while the figures for aluminium and iron phosphates are 191 and 289.9. The amount of hydrolysis in the case of aluminium phosphate and ferric phosphate is less rapid than in tricalcium phosphate solution. This is more obvious from the observation that slightly moistened tricalcium phosphate behaves alkaline towards litmus while a solution of tricalcium phosphate is distinctly on the acidic side. This marked difference between the two groups of phosphates regarding their amounts of hydrolysis can be attributed to the adsorptive powers of  $Al_2O_3$  and  $Fe_2O_3$  for phosphoric acid which do not allow much

phosphoric acid to remain in the solution than in the case of tricalcium phosphate system. At 30°C the amount of decomposition of aluminium phosphate is greater than ferric phosphate but at 50°C ferric phosphate suffered greater decomposition than aluminium phosphate.

It appears that as the fall of pH for the three phosphates is nearly same at 30°C. It indicates that they give out hydrogen ions in the solution to the same extent. But on the other hand the tricalcium phosphate solution shows marked change in conductivity as compared to aluminium phosphate and ferric phosphate solutions. From this we infer that ferric phosphate and aluminium phosphate will produce hydrogen ions much more readily than phosphate ions whilst tricalcium phosphate may be able to give out both phosphate ions and hydrogen ions but ferric phosphate will give out hydrogen ions but not much phosphate ions.

There is no doubt that in the case of ferric and other heavy metal phosphates complex acids giving out hydrogen ions and ferro phosphate ions may be produced more readily than in the case of calcium or other phosphates. This seems to be an essential difference between the two group of phosphates.

Originally a solution of tricalcium phosphate is more conducting than ferric phosphate solution, hence in tricalcium phosphate solutions in soils both calcium and phosphates are available while in the case of ferric phosphate only hydrogen ions are available and small amounts of phosphate ions.

The dicalcium phosphate solution suffered very little decomposition at 30°C as well at 50°C. It is interesting to note here that the di salt showed a very peculiar behaviour in the way that at 30°C firstly the conductivity increased for about 6 hours probably due to dissolution of the salt and partial hydrolysis, then it decreased for about 30 days and again increased. The decrease in conductivity during the course of this change can most probably be attributed to adsorption of the ions by the surface. At 50°C it decreased for the first 21 hours and then increased continuously. The increase of conductivity and decrease in pH indicate that the salt is hydrolysed to give acid in solution and a more basic compound in the solid phase. The dimagnesium phosphate behaves similarly. Decomposition of this salt is, however, smaller than aluminium, ferric and calcium phosphates.

The monocalcium phosphate suffered very little change at 30° and a slight change at 50°C. Analysis of the solid residue indicates that it is mainly the di salt. Hence it appears that the mono salt is immediately decomposed into the di salt and then very little change occurs. It is most probable that the changes in conductivity of the mono salt at 50° may be attributed to the hydrolysis of the di salt which is formed immediately when the mono salt comes into contact with water.

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## PHOSPHATE FIXATION IN LATERITE SOILS OF MADRAS

By T. RAJAGOPALA IYENGAR, K. SHIVARAMA SHETTY and D. JOHN DURAIRAJ

*Agricultural College and Research Institute, Coimbatore*

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### INTRODUCTION

The problem of phosphate fertilization, unlike that of the other major plant nutrients, is considerably complicated as it is involved with a variety of soil factors. The one single factor which plays a vital role in this matter is probably soil pH. If this is too high the presence of considerable amounts of calcium and magnesium in the soil is indicated and this brings about the conversion of applied soluble phosphates to comparatively insoluble forms. With a low pH below 5.0, the soluble phosphates added to such soils is fixed or rendered unavailable by the formation of iron and aluminium phosphates. The latter condition is prevalent in the laterite soil rich in iron and alumina and poor in plant nutrients. The major portion of these elements (Fe and Al) exist as their hydrated oxides, in which form they have a pronounced affinity for phosphates — the prevailing low pH rendering the formation of iron or aluminium phosphates a comparatively easy matter. The fixation of phosphate in acid soils takes various forms<sup>1,2</sup> under different pH levels. If the pH is of the order of 5, a comparatively weak "fixation" occurs, by the displacement of OH ions in the crystal lattice by  $\text{PO}_4$  ions. Only if the pH is as low as 3 chemical "fixation" takes place; this represents a permanent and irreversible form of "fixation" and the recovery of phosphate from this form is very difficult. Fortunately, the pH of the laterite soils under study seldom falls below 4.5, and hence, the possibility of the recovery of "fixed" phosphate is a fairly feasible proposition by adopting suitable ameliorative measures.

In a soil system, suffering from so many inherent defects with reference to phosphate fertilization, the use of phosphatic manures becomes wasteful and uneconomical. Thus the problem has engaged the close attention and interest of soil workers, who have deduced certain broad principles applicable to profitable use of phosphatic fertilizers.

### LITERATURE

The incomplete availability of superphosphate applied to the soil engaged the attention of Gerlach<sup>3</sup> as early as 1895; by experimenting with solutions of monocalcium phosphate and superphosphate, and a suspension of ferric hydroxide in water, he was able to prove that the phosphates applied to the soil were "fixed" as the phosphates of Ca, Mg, Fe and Al. Ellett and Hill<sup>4</sup> later on demonstrated the low solubility of phosphates fixed by Al and Fe hydroxides, by mixing these hydroxides with water solutions of monocalcium phosphate and superphosphate and extracting the dried mixtures with 0.2 N

HNO<sub>3</sub>; only 50% of the phosphate could now be extracted with the nitric acid.

Birch<sup>3</sup> reviewing the work on the fixation of phosphates in soil mentioned that the chief factors affecting this phenomenon were clay content, hydroxide content, and pH of the soil. Among the principal method employed for securing maximum utilisation of applied phosphate, he mentioned liming, placement of fertilizer, pre-treatment of seeds with phosphate solutions, application of organic manures and the use of sodium silicate.

Fraps<sup>8</sup> observed a close correlation between the phosphoric acid absorbed, and the acid soluble sesquioxides of the soil. Heck and Troug<sup>10</sup> studying the effect of application of phosphate to soils rich in lime or sesquioxides, concluded that in the former instance, the phosphate was converted into tricalcium phosphate, a comparatively available form of phosphate, while in the latter instance the phosphate was "fixed" as basic iron and aluminium phosphates. Bartholomew and Jacob<sup>2</sup> assessing the relative availability of aluminium and iron phosphates by studying their effect on Sudan grass, observed that keeping the value for monocalcium phosphate as 100, those for unignited aluminium and ferric phosphates were 42.8 and 8.4 respectively.

Toth<sup>12</sup> investigating the mechanism of phosphate fixation, found that the main reactions involved were (a) replacement of lattice OH ions or "broken band" OH ions by PO<sub>4</sub>, (b) replacement of silicate ions by PO<sub>4</sub>; (c) precipitation on the colloidal surfaces as insoluble phosphate (chemisorption); and (d) precipitation as Ca, Mg, Fe, Al and Mn phosphates. In a similar investigation, Moser<sup>11</sup> adduced evidence to show that phosphate fixation was brought about by (a) adsorption by clay particles, (b) formation of a silico-phospho-aluminium complex, and (c) replacement of OH ions within the crystal lattice by PO<sub>4</sub> ions.

Davis and Brewer<sup>5</sup> pointed out that liming of soils low in calcium content enabled crops to utilise larger quantities of phosphorus supplied as superphosphate. Barbier, Maroger and Gachore<sup>1</sup> observed that the humic anion in the ferric humus complex reduced phosphate fixation by iron, and the organic manure considerably increased the acid soluble mineral fraction of soil phosphorus. Considerable increases in the availability of fixed phosphorus in the presence of organic compounds, such as, sucrose, glucose, or pectin, were observed by Dalton, Russell, and Sieling<sup>4</sup> Doughty<sup>6</sup> showed that Fe and Al phosphates hydrolyse at a reaction range of pH 6.0 to 7.0, releasing phosphorus to the soil solution.

#### STUDIES ON PHOSPHATE AVAILABILITY IN MADRAS LATERITE SOILS

A scheme of studies for observing the mechanism of phosphate fixation and availability, and for fixing correct ameliorants and their doses, was initiated at Agricultural Research Station, Pattambi (Malabar District) in July 1951 and conducted for three years.

#### EXPERIMENTS

Preliminary laboratory scale experiments were conducted at Coimbatore for the study of reversion of phosphate supplied as rock phosphate or superphosphate. Field experiments with 36 treatments embodying various combinations of superphosphate, lime and green leaf dosages were conducted at Pattambi,

during 6 crops seasons, on wetland paddy. Pot culture experiments on paddy were conducted during three crop seasons at Pattambi, employing 13 treatments representing combinations of lime, green leaf, superphosphate, rock phosphate, bone meal and ammonium sulphate. Soil, sub-soil, grain and straw samples collected during the field experiments were subjected to chemical analysis. Statistical analysis of grain and straw yield data from the field and pot culture experiments was performed.

## RESULTS

1. *Preliminary Experiments.* Information collected from these experiments indicate that, (a) ferric hydroxide is more powerful in "reverting" phosphates than aluminium hydroxide, (b) superphosphate is a more available form of phosphate than rock phosphate, and (c) lime and green leaf increase the availability of the phosphate from these two phosphates and are effective in checking reversion.

The mechanism of action of lime is probably dependent upon the raising of soil pH temporarily bringing about thereby increased availability of soluble phosphate. Moreover the growth of bacteria responsible for the decomposition of any freshly added organic material and the formation of simple and easily available nitrogenous and phosphatic compounds during the process is favourable during the rise of pH, by the plant. The production of mild organic acids during decomposition of the green manures will also protect the soluble phosphate and prevent it from getting "fixed" as iron and aluminium phosphates.

(2) *Field Experiments.*--Table 1 shows the degree of significance of yield data, and the effect of ameliorants on the yield with passage of time. The ranking of treatments has been almost the same for grain and straw. In the case of grain, green leaf and lime were the chief determining factors, with reference to yield, at the beginning; during three of the succeeding crops, the importance of lime decreased considerably; the last experiment of the 6-crops series indicated that the lowest dose of superphosphate (30 lb  $P_2O_5$  per acre) and the lower dose of green leaf (5000 lb per acre) were fairly satisfactory with reference to yields. Straw yields showed that the effect of superphosphate was prominent at the beginning; thereafter combinations of super and green leaf gained prominence; Lime is not observed to be as important as it was in the case of grain.

In the majority of cases, no statistical interactions between the various treatments were observed, indicating that the treatment acted independently of one another.

Table 3 brings out the relative importance of the various applications and their doses. Similar responses were registered by grain and straw. The 1500 and 3000 lb doses of lime produced almost proportionate increases in yield; the lower dose of green leaf (5000 lb) produced little beneficial effect, while the higher dose (7500 lb) had a marked effect in increasing the yield; in the case of superphosphate there was little difference between the responses in yield for the three doses (30, 45 and 60 lb  $P_2O_5$ ).

(3) *Pot Culture Experiments, (Table 2).* The statistical analysis of grain and straw yield data indicated high significance. The treatment which was best with reference to yield was observed to be the combination of doses of lime, green leaf and superphosphate corresponding to 3000 lb, 7500 lb and 60 lb  $P_2O_5$  per acre respectively. Most of the best performing treatments were observed to contain the

7500 lb dose of green leaf in combination. Lime was found to increase the availability of superphosphate and rock phosphate.

(4) *Chemical Analysis.* Table 4 gives a general idea of the effect of the various treatments employed in the field experiments on the principal chemical constituents of the soil.

The comparative study of the chemical composition of the soil samples collected after each field experiment showed that the loss on ignition, lime and total and available  $P_2O_5$  values for the soil increased during the first two crop seasons. Total  $P_2O_5$  and nitrogen values became steady at this point, but the available  $P_2O_5$ , exchangeable calcium and pH continued to increase till the end of the second year. The values were observed to have become steady during the third year.

In the case of grain, the nitrogen content was found to be increased slightly by green leaf or lime, and decreased slightly by  $P_2O_5$  doses. The phosphorus and calcium contents were increased by increasing superphosphate and lime doses, respectively. Analysis of straw indicated that its phosphorus content is dependent upon the dose of superphosphate applied.

#### DISCUSSIONS

The probable reactions involved in the beneficial action of lime and green leaf in rendering superphosphate more available and preventing its reversion, are as follows:—The lime brings about a temporary rise in pH; with the reaction thus approaching near-neutrality, the availability of phosphate is considerably increased, and chances of phosphate reversion rendered less. Moreover, under these optimum conditions of soil microbial activity, the breaking down of nitrogenous organic matter into simpler, assimilable forms by microbes takes place and the availability of nitrogen considerably increased. The applied green manure is decomposed by the microbes with the formation of mild acids, which effectively prevent the reversion of applied soluble phosphate.

#### CONCLUSIONS

Superphosphate is superior to rock phosphate as a source of readily available phosphate with regard to growth and grain production in paddy under wetland cultivation. Green leaf and lime applied along with superphosphate increase its availability considerably. The full magnitude of the beneficial effect of lime is felt during the first two seasons; thereafter lower doses of this ameliorant are sufficient for good crop response. The high dose of green leaf (7500 lb) is much more effective than the lower one (5000 lb). The full dose of this ameliorant is indispensable in maintaining the availability of applied phosphate.

The following scheme may be adopted for increasing the availability of superphosphate and securing good yields of paddy in laterite soils of Madras, under wetland cultivation: Superphosphate, lime and green leaf are to be applied at the rate of 60 lb  $P_2O_5$ , 3000 lb and 7500 lb per acre respectively during the first two crop seasons. Thereafter, the doses of superphosphate and lime alone are reduced to 30 lb  $P_2O_5$  and 1500 lb respectively, retaining the original dose of green leaf.

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## Appendix Tables

### TABLE 1

MANURIAL EXPERIMENTS ON LATERITE SOILS—AGRICULTURAL RESEARCH STATION,  
PATIAMB, FIELD EXPERIMENTS, 1951-52

I Crop to 1953-54 II Crop

Particulars		1951-52		1952-53		1953-54	
1. 'F' value for significance of yield data (Theoretical 'F' value at 5% level: 1.25)							
	Grain	0.94	14.73	6.69	6.42	5.04	2.81
	Straw	2.86	11.8	5.00	5.28	3.35	2.89
2. Acre yield in lb							
1. Maximum	Grain	386.0	1998	2144	1801	3256	1335
	Straw	1900	2432	4303	4452	8744	7375
2. Minimum	Grain	54.2	342.4	894	580	1897	803
	Straw	1062	614.9	1928	1677	4638	3995
3. Control	Grain	151.9	356.3	894	580	1897	803
	Straw	1257	642.8	1928	1677	4638	3995
4. General mean	Grain	15.11	1107	1706	1347	2759	1131
	Straw	15.1	1541	3324	3277	6939	5985

### TABLE 2

MANURIAL EXPERIMENTS ON LATERITE SOILS—AGRICULTURAL RESEARCH STATION,  
PATIAMB, POT CULTURE EXPERIMENTS, 1952-53

II Crop to 1953-54 II Crop

Particulars		1952-53 II Crop*	1953-54 I Crop	1953-54 II Crop
1. 'F' values for significance of yield data (Theoretical 'F' value at 5% level = 2.00)				
	Grain	4.14	10.54	5.91
	Straw	...	5.79	11.74
2. Acre yield per plant in grams				
1. Maximum	{ Grain	4.002*	3.456	2.168
	{ Straw		1.908	2.270
2. Minimum	{ Grain	1.290*	1.423	1.082
	{ Straw		0.800	1.054
3. Control	{ Grain	1.596*	1.432	5.216
	{ Straw		0.800	1.112
4. General mean	{ Grain	2.303*	2.297	1.689
	{ Straw		1.303	1.661

\* Data "per plot."

TABLE No. 3

MANURIAL EXPERIMENT ON LATURITE SOILS, AGRICULTURAL RESEARCH STATION, PATTAMBI, FIELD EXPERIMENTS,  
1951-52—I CROP TO 1953-54 II CROP

No.	Treatments and doses	Mean Yield per plot for Treatment									
		1951-52					1952-53				
		I Crop grain oz	I Crop straw lb	II Crop grain oz	II Crop straw lb	I Crop grain oz	I Crop straw lb	II Crop grain oz	II Crop straw lb	I Crop grain oz	II Crop straw lb
1.	Lime										
	(a) 0 lb	...	12.85	89.21	9.27	228.4	28.23	168.8	25.61	373.5	58.99
	(b) 1500 lb	...	13.48	160.6	14.54	242.2	29.56	196.7	29.96	396.6	62.34
	(c) 3000 lb	...	14.23	225.4	17.71	262.5	31.12	213.2	322.5	415.9	64.98
2.	Leaf										
	(a) 0 lb	...	13.65	133.2	12.02	220.7	27.20	161.3	25.42	365.5	55.23
	(b) 5000 lb	...	13.38	139.8	12.44	247.7	29.34	191.6	28.63	397.1	63.57
	(c) 7500 lb	...	13.54	202.1	17.06	264.7	32.21	230.9	33.94	423.0	67.60
3.	Super P <sub>2</sub> O <sub>5</sub>										
	(a) 0 lb	...	11.95	150.8	12.31	188.8	23.29	149.3	25.25	350.9	54.62
	(b) 30 lb	...	12.94	159.4	1.397	257.2	30.83	197.7	29.18	400.5	64.34
	(c) 45 lb	...	14.31	156.9	14.08	262.0	31.81	210.6	30.22	412.3	64.73
	(d) 60 lb	...	14.89	166.5	14.11	269.3	33.09	214.0	31.75	416.7	64.65
											57.84



TABLE 4

RESULTS OF CHEMICAL ANALYSIS OF SOILS—1952-53—II CROP—PERCENTAGES : MOISTURE-FREE BASIS

Lime lb		0										1500														
Green leaf		0					5000					7500					0					5000				
$P_2O_5$ (as super)		0	30	45	60	0	30	45	60	0	30	45	60	0	30	45	60	0	30	45	60					
Dosage numbers		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20					
1. Moisture		2.06	2.26	3.32	2.32	2.61	1.78	2.63	2.36	2.54	2.61	2.37	2.21	2.31	2.28	2.43	2.28	2.50	2.47	2.10	2.10					
2. Total $P_2O_5$		.131	.174	.167	.182	.189	.144	.173	.188	.180	.198	.188	.199	.172	.193	.181	.182	.183	.186	.206	.231					
3. Available $P_2O_5$ ( $\times 10,000$ )		12	19	29	41	19	17	29	44	24	47	56	51	11	38	35	55	30	48	45	71					
4. Nitrogen		.232	.219	.226	.219	.286	.178	.269	.252	.277	.299	.308	.293	.232	.223	.212	.227	.260	.244	.276	.253					
5. Exchangeable Ca (m. e. %)		1.3	2.1	1.2	2.0	3.5	2.6	2.2	2.3	3.0	3.1	2.7	2.2	3.6	4.5	4.1	4.2	3.9	4.5	3.4	4.1					
6. pH		5.0	5.1	4.8	5.0	5.3	6.1	5.7	5.6	5.3	5.3	5.1	5.0	6.5	5.8	5.9	7.0	5.5	6.0	5.4	5.4					
Lime		1500										3000														
Green leaf		7500					0					5000					7500									
$P_2O_5$ (as super)		0	30	40	60	0	30	40	60	0	30	40	60	0	30	40	60	0	30	40	60					
Dosage No.		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36									
1. Moisture		2.48	2.34	2.24	1.47	2.09	2.35	2.50	2.16	2.87	2.56	2.04	2.28	2.34	1.77	1.89	2.18									
2. Total $P_2O_5$		.171	.186	.228	.180	.164	.171	.195	.194	.162	.190	.194	.202	.182	.204	.206	.217									
3. Available $P_2O_5$ ( $\times 10,000$ )		17	55	71	59	26	50	60	56	17	68	49	40	36	71	67	74									
4. Nitrogen		.276	.257	.272	.216	.217	.201	.224	.315	.239	.244	.216	.246	.211	.267	.239	.258									
5. Exchangeable Ca (m. e. %)		3.9	4.1	3.6	3.7	6.7	8.3	10.5	9.5	4.0	7.0	7.0	5.4	7.2	6.0	6.0	6.4									
6. pH		5.3	6.3	5.8	6.3	6.8	6.5	7.1	6.7	5.5	6.3	6.2	6.0	6.4	6.5	6.6	6.7									

# VALUE OF DIFFERENT PHOSPHATES IN NITROGEN FIXATION IN SOILS

By N. R. DHAR and B. K. DHAR

*Sheila Dhar Institute of Soil Science, University of Allahabad.*

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Recently Dhar<sup>1</sup> has emphasized in many of his publications that soils rich in phosphates can be rich in nitrogen also. He is of the opinion that phosphates when mixed with organic substances like green manure, farmyard manure, straw, leaves, grass and other plant residues can act as partial substitutes for nitrogen fertilizers by fixing atmospheric nitrogen and supply available phosphate, potash and trace elements and build up soil fertility by increasing the humus status.

Bear<sup>2</sup> has also stated that "within limits phosphate fertilizers together with potash salts and lime can be substituted for nitrogen fertilizers. Their use stimulates the nitrogen fixing bacteria, both symbiotic and non-symbiotic to greater activity."

In view of the above observations we have studied the influence of monocalcium, dicalcium phosphate, tricalcium phosphate, rock phosphate, superphosphate and the phosphates of iron, aluminium and magnesium on the nitrogen fixation effected by the oxidation of energy rich materials such as molasses and wheat straw when applied to different soils. The phosphates used in these experiments were first tested for their purity. Rock phosphates were obtained from different parts of India and the superphosphates were prepared by the authors in the laboratory by adding different amounts of sulphuric acid to definite weights of rock phosphates collected from India.

## EXPERIMENTAL PROCEDURE

0.25 %  $P_2O_5$  in the form of above mentioned compound of phosphates were added to two Indian soils. The moisture content of all the experiments was maintained at 20 %. After definite intervals of time the soil samples were taken out for the analysis of total carbon and total nitrogen. The total carbon was determined by the method of Robinson, Mclean and Williams,<sup>3</sup> and the total nitrogen was estimated by salicylic acid reduction method.<sup>4</sup>

### *Analysis of the normal soil*

Loss on ignition	= 4.128 %	$Fe_2O_3$	= 4.01 %
HCl insoluble	= 81.82 %	CaO	= 1.002 %
Sesquioxide	= 8.52 %	MgO	= 1.759 %
$P_2O_5$	= 0.079 %	$K_2O$	= 1.021 %
Available $P_2O_5$	= 0.0329 %	Exchangable calcium }	= 22.1 m.e
Total carbon	= 0.4012 %	Total nitrogen	= 0.0422 %
pH = 7.6			

# *Analysis of molasses*

Total carbon	=	81.52 %	Total nitrogen	=	0.525 %
Silica	=	6.120 %	CaO	=	1.35 %
P <sub>2</sub> O <sub>5</sub>	=	0.0603 %	MgO	=	0.001 %

## RESULTS

Average Temp. 30°C

Period of exposure in days	Total carbon	Carbon oxidized	Total Nitrogen	Nitrogen fixed	Efficiency i.e., amount of nitrogen fixed in mgs per gram of carbon oxidized
200 grms of soil + 2 grms of Molasses					
Light					
0	0.7093	...	0.04700	...	...
50	0.4321	0.2772	0.05925	0.01225	44.2
100	0.4107	0.2986	0.06133	0.01433	48.0
Dark					
0	0.7093	...	0.04700	...	...
50	0.4402	0.2691	0.0534	0.00640	23.8
100	6.4299	0.2794	0.0539	0.00698	25.0
200 grms of soil + 2 grm Molasses + 0.25 % P <sub>2</sub> O <sub>5</sub> as Phosphate rock Trichinopoly					
Light					
0	0.7100	...	0.04760	...	...
50	0.4231	0.2869	0.06217	0.01457	50.8
100	0.4019	0.3081	0.06451	0.01691	54.9
Dark					
0	0.7100	...	0.04760	...	...
50	0.4390	0.2710	0.0550	0.00742	26.9
100	0.4269	0.2831	0.0564	0.00886	31.3
200 grms soil + 2 grms Molasses + 0.25% P <sub>2</sub> O <sub>5</sub> as Phosphate rock of Behar					
Light					
0	0.7099	...	0.0475	...	...
50	0.4228	0.2871	0.0621	0.01469	51.2
100	0.4019	0.3080	0.0645	0.01706	55.4
Dark					
0	0.7099	...	0.0475	...	...
50	0.4375	0.2724	0.0545	0.00702	27.4
100	0.4265	0.2834	0.0559	0.00841	29.7

Period of exposure in days	Total carbon	Carbon oxidized	Total nitrogen	Nitrogen fixed	Efficiency i.e., amount of nitrogen fixed in mgs per gram of carbon oxidized
200 grms of soil + 2 grms molasses + 0.25% $P_2O_5$ as Monocalcium phosphate $Ca(H_2PO_4)_2 \cdot H_2O$					
Light					
0	0.7092	...	0.0467	...	...
50	0.4229	0.2863	0.0606	0.01397	48.8
100	0.4053	0.3039	0.0627	0.01609	52.9
Dark					
0	0.7092	...	0.0467	...	...
50	0.4301	0.2782	0.0551	0.00840	30.4
100	0.4272	0.2820	0.0556	0.00854	31.4
200 grms of soil + 2 grms Molasses + 0.25% $P_2O_5$ as Dicalcium Phosphate $CaHPO_4 \cdot 2H_2O$					
Light					
0	0.7092	...	0.0468	...	...
50	0.4216	0.2876	0.0620	0.01522	52.6
100	0.4006	0.3086	0.0644	0.01765	57.2
Dark					
0	0.7092	...	0.0468	...	...
50	0.4369	0.2723	0.05483	0.00803	29.5
100	0.4360	0.2832	0.0553	0.00855	30.2
200 grms of soil + 2 grm Molasses + 0.25% $P_2O_5$ as Tricalcium phosphate $Ca_3(PO_4)_2$					
Light					
0	0.7092	...	0.0470	...	...
50	0.4230	0.2862	0.0619	0.01493	52.2
100	0.4011	0.3081	0.0645	0.01751	56.9
Dark					
0	0.7092	...	0.0470	...	...
50	0.4365	0.2728	0.0544	0.00742	27.2
100	0.4266	0.2826	0.0554	0.00842	29.8
200 grms of soil + 2 grms Molasses + 0.25% $P_2O_5$ as Superphosphate with 2 mols $H_2SO_4$					
Light					
0	0.7093	...	0.0469	...	...
50	0.4196	0.2897	0.0608	0.01392	46.9
100	0.4002	0.3091	0.0621	0.01523	49.3

Period of exposure in days	Total carbon	Carbon oxidized	Total nitrogen	Nitrogen fixed	Efficiency i.e., amount of nitrogen fixed in mgs per gram of carbon oxidized
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Dark

0	0.7093	...	0.0469	...	...
50	0.4299	0.2794	0.0541	0.0072	25.8
100	0.4206	0.2887	0.0556	0.00871	30.2

200 grms soil + 2 grms Molasses + 0.25%  $P_2O_5$  as Superphosphate with  
1 mol  $H_2SO_4$

Light

0	0.7093	...	0.0470	...	...
50	0.4165	0.2928	0.0610	0.0140	47.8
100	0.4011	0.3082	0.0621	0.01516	49.2

Dark

0	0.7093	...	0.0470	...	...
50	0.4382	0.2711	0.0541	0.00715	26.4
100	0.4241	0.2852	0.0551	0.00815	28.6

200 grms soil + 2 grms Molasses + 0.25%  $P_2O_5$  as Superphosphate with  
mol  $H_2SO_4$

Light

0	0.7093	...	0.0470	...	...
50	0.4210	0.2903	0.0613	0.0143	48.9
100	0.4010	0.3083	0.0631	0.01650	52.4

Dark

0	0.7093	...	0.0470	...	...
50	0.4388	0.2705	0.0538	0.00684	25.3
100	0.4258	0.2835	0.0545	0.00750	26.6

200 grms soil + 2 grms Molasses + 0.25%  $P_2O_5$  as Aluminium phosphate

Light

0	0.7093	...	0.0470	...	...
50	0.4298	0.2795	0.0598	0.01288	46.1
100	0.4093	0.3000	0.0617	0.01476	49.2

Dark

0	0.7093	...	0.0470	...	...
50	0.4492	0.2601	0.0536	0.00660	25.4
100	0.4295	0.2789	0.0545	0.00750	27.7

Period of exposure in days	Total carbon	Carbon oxidized	Total nitrogen	Nitrogen fixed	Efficiency i.e., amount of nitrogen fixed in mgs per gram of carbon oxidized
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200 grm soil + 2 grms Molasses + 0.25%  $P_2O_5$  as Ferric phosphate

Light					
0	0.7093	...	0.0471	...	...
50	0.4301	0.2791	0.0601	0.01306	46.8
100	0.4094	0.2993	0.0620	0.01500	50.1
Dark					
0	0.7093	...	0.0471	...	...
50	0.4400	0.2692	0.0537	0.00667	24.8
100	0.4292	0.2800	0.0547	0.00762	27.4

Experiments with good soil using wheat straw as an energy material

*Analysis of the soil*

Loss on ignition =	6.380%	$Fe_2O_3$ =	4.575 %
HCl insoluble =	74.330%	CaO =	4.12 %
Sesquioxides =	9.730%	$P_2O_5$ =	0.412 %
$K_2O$ =	0.983%	MgO =	1.81 %
Total carbon =	2.00 %	Total Nitrogen =	0.256

pH = 8.1

*Analysis of straw*

Ash =	8.620%	$P_2O_5$ =	0.052%
Silica =	6.520%	MgO =	0.56 %
CaO =	0.285%	$K_2O$ =	0.61 %
Total carbon =	39.23%	Total nitrogen =	0.734%

Treatment

Efficiency

	After 90 days exposure		After 180 days exposure	
	Light	Dark	Light	Dark
Soil + 1% wheat straw	135.0	70.1	130.2	73.4
Soil + „ + phosphate rock of Behar	148.3	85.0	144.3	86.0
Soil + „ + phosphate rock of trichinopoly	150.3	75.0	148.2	76.6
Soil + „ + monocalcium phosphate	148.6	86.9	147.5	89.6
Soil + „ + dicalcium phosphate	155.8	88.4	151.7	89.6
Soil + „ + tricalcium phosphate	155.2	85.4	154.1	86.5
Soil + „ + superphosphate with 2 mols $H_2SO_4$	128.0	60.5	125.0	63.7
Soil + „ + superphosphate with 1 mol $H_2SO_4$	182.4	69.3	130.2	72.4
Soil + „ + superphosphate with $\frac{1}{2}$ mol $H_2SO_4$	137.5	72.5	134.4	70.5
Soil + „ + aluminium phosphate	138.1	72.8	132.4	77.6
Soil + „ + ferric phosphate	136.9	69.2	134.6	63.1

The foregoing results show that when molasses is allowed to undergo slow oxidation in air in contact with soil there is always a decrease in carbon content and a concomitant increase in the nitrogen content of the system and the effi-

ency (nitrogen fixed in mgrms. per gram of carbon oxidized). This effect is more pronounced in light than in the dark. When phosphates are added there is a greater increase of nitrogen than in their absence. The increase in the case of iron and aluminium phosphate is smaller than with monocalcium, dicalcium, tricalcium phosphates. When rock phosphates obtained from different parts of India are added to the system they produce beneficial results towards nitrogen fixation. It is interesting to note that monocalcium phosphate which is distinctly acidic is not as effective in the increase of nitrogen as the dicalcium and tricalcium phosphates. Similarly superphosphate prepared by the author by the action of sulphuric acid on Indian phosphate rocks is also less beneficial than the dicalcium and tricalcium phosphates as such.

From the experiments carried out with good quality of soil using straw as a source of energy it has been observed that there is a very high nitrogen fixation in the soil. This is due to the fact that this soil contains 4.1% CaO and 0.40%  $P_2O_5$  and is rich in calcium phosphates. Here also we see that the addition of different phosphates enhances the nitrogen fixation.

It is generally believed that the phenomenon of nitrogen fixation in soil is essentially a bacterial process. Dhar and co-workers<sup>5,7</sup> have, however, shown in a number of publications that when different energy producing materials such as sugars, glycerol, starch, fats, cellulose, straw, saw-dust, leaves, plant, peat, lignite, etc. undergo slow oxidation in air in presence of soil, sand or any other chemically pure substance like  $Fe_2O_3$ ,  $Ni_2O_3$ ,  $MnO_2$ ,  $ZnO$ , etc. as surface under completely sterile conditions, there is always fixation of atmospheric nitrogen which is always more pronounced in light than in the dark. Our experiments also show that there is appreciable carbon oxidation and concomitant increase in the total nitrogen of the system, when energy materials like molasses and straw are allowed to undergo slow oxidation in air. These results are in complete agreement with the conclusions of Dhar and co-workers.

The increase of nitrogen in the system has been attributed to the fact when organic matter is added to the soil it undergoes slow oxidation and liberates energy which fixes the atmospheric nitrogen in the soils. When the light acts on the system whether from the sun or artificial source a part of it is absorbed and causes greater fixation of nitrogen.

Dhar in his Presidential Address to the National Academy of Sciences, India, in 1953, emphasized from a survey of world soils that the nitrogen status of the world soils depends a good deal on their phosphate status as soils rich in phosphate can also be rich in nitrogen when organic substances are present therein. Recently Thompson<sup>8</sup> has reported that a close relationship exists between total nitrogen and  $P_2O_5$  in 50 soils from Texas, Colorado and Iowa. His results are recorded in the following table:—

No. of soils in each group	Total phosphorus p. p. m.	Total nitrogen average %
2	800—999	0.375
25	600—799	0.325
17	400—599	0.226
4	300—399	0.151
2	0—199	0.111

The above table clearly shows that greater the total  $P_2O_5$ , greater is the total nitrogen in the soil, supporting the conclusions of Dhar and co-workers.

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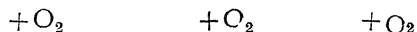
# RETARDING INFLUENCE OF PHOSPHATES ON NITROGEN LOSS FROM SOILS

By N. R. DHAR and B. K. DHAR

*Sheila Dhar Institute of Soil Science, University of Allahabad*

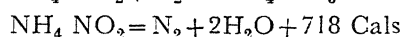
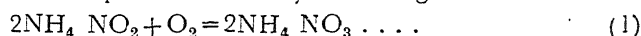
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Dhar and co-workers<sup>1</sup> have proved that when nitrogenous fertilizers or proteins are added to the soil under aerobic condition, the following take place :—



Proteins—→Amino acids—→ammonia—→nitrite—→nitrate

These changes are oxidation processes which are accelerated by increased aeration, absorption of solar light and by increase of temperature. In these processes an unstable intermediate compound ammonium nitrite is formed which can undergo both oxidation and decomposition aided by the sunlight as follows :—



The second chemical change is more prominent than the first as it is highly exothermic and hence there is a considerable loss of nitrogen from soils when manured with all types of nitrogenous compounds. The temperature coefficient of the decomposition of ammonium nitrite has been found by Arndt<sup>2</sup> to be of the order of 3 for a 10°C rise of temperature so that the reaction seems to be prominent in tropical soils. Again the decomposition of ammonium nitrite is an autocatalytic process and is markedly accelerated by acids so that the loss of nitrogen also becomes prominent in cold countries, where the soils have a tendency to be acidic, and specially in soils not containing much calcium carbonate or calcium phosphate.

Field trials have shown that the recovery of nitrogen by crops never exceeds 50% whilst the recovery of phosphate and potash may go up to 85%. Löhnis and Fred<sup>3</sup> have reported the following recovery in field experiments lasting for 4 years :—

Nitrogen	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
7.8 to 46.1%	10.1 to 75.6%	22.4 to 85.1%

One of the synthetic fertilizers commonly used is ammonium sulphate. Russell<sup>4</sup> has stated that the recovery of nitrogen from it when added at the rate of 1 cwt per acre is as follows :—

Crops	Average nitrogen recovery
Wheat	39.0%
Barley	47.5%
Oats	46.5%
Patatoes	50.0%
Swedes	35.5%

The researches of Lipman and Blair<sup>5</sup>, Russell and Richards<sup>6</sup>, Shult<sup>7</sup> and others have shown that nitrogen in the gaseous state is lost from soils when the conditions are favourable for oxidation.

In view of the above observations we have investigated the influence of adding different calcium phosphates to ammonium sulphate in small doses, in the form of superphosphate, rock phosphate, and basic slag obtained from steel industry of India and bone meal.

#### EXPERIMENTAL PROCEDURE

To the soil 0.03% nitrogen in the form of ammonium sulphate was added. Also 0.10%  $P_2O_5$  in the form of monocalcium phosphate, dicalcium phosphate, tricalcium phosphate, bone meal, basic slag, and different types of superphosphates (prepared by the authors in the laboratory) and rock phosphate collected from different parts of India were added to the mixtures of soil and ammonium sulphate. The moisture content of the system was maintained at 20%.

After definite intervals of time the soil samples were taken out and analysed for total carbon by Robinson, Williams and Mclean's<sup>8</sup> method, total nitrogen by the salicylic acid reduction method and  $NH_3$ -nitrogen and  $NO_3$ -nitrogen were determined by MgO method and Devard's alloy reduction method respectively.

The analysis of the soil used is as follows :—

Loss on ignition	=4.128	%	HCl insoluble	= 81.82	%
Sesquioxides	=8.520	%	$Fe_2O_3$	= 4.01	%
CaO	=1.002	%	$P_2O_5$	= 0.0791	%
MgO	=1.759	%	$K_2O$	= 1.021	%
Total carbon	=0.4012	%	Total nitrogen	= 0.0422	%
$NH_3$ -nitrogen	=0.01122	%	$NO_3$ -Nitrogen	=0.002080	%

#### EXPERIMENTAL RESULTS

The average temperature of the system in these experiments was 32°C.

*Percentage loss of nitrogen from Ammonium sulphate and comparative effect of different phosphate on the loss of nitrogen*

Treatment	Percentage loss of nitrogen from Ammonium sulphate			
	After 40 days		After 80 days	
	Exposed	Kept in dark	Exposed	Kept in dark
1. Ammonium sulphate	56.2	50.2	69.5	62.5
2. „ + monocalcium phosphate	49.0	44.0	52.0	46.5
3. „ + dicalcium phosphate	45.0	41.0	50.0	45.0
4. „ + tricalcium phosphate	42.0	38.2	49.0	45.0
5. „ + phosphate rock (kudada)	41.5	37.2	49.2	41.8
6. „ + phosphate rock of trichiopoly	42.3	38.0	49.0	44.0
7. „ + phosphate rock of Behar	41.0	37.3	48.3	44.4
8. „ + bone meal	39.0	35.6	46.6	41.4
9. „ + basic slag	38.6	37.6	48.3	44.0
10. „ + superphosphate with 2 mols $H_2SO_4$	48.3	45.0	52.0	47.8
11. „ + superphosphate with 1 mol $H_2SO_4$	48.6	44.3	51.6	47.0
12. „ + superphosphate with $\frac{1}{2}$ mol $H_2SO_4$	47.0	42.0	50.0	45.3

The results recorded above show that when ammonium sulphate is added to the soil there is always a loss of nitrogen in the gaseous state attended with the formation of nitrites. But when ammonium sulphate is added to the soil with different phosphates as recorded in the foregoing results, the loss of nitrogen from ammonium sulphate is appreciably checked. It has always been observed that the loss of nitrogen is always more prominent in the sets exposed to light than those kept in the dark. The loss of nitrogen from ammonium sulphate is checked to a greater extent in presence of monocalcium, dicalcium, tricalcium and rock phosphates and bone meal.

It is well-known that when ammonium salts are added to the soil they are oxidized to nitrites and nitrates before they are utilized by plants. It is generally believed that the nitrification in soils is essentially due to the action of different kinds of soil micro-organisms. The biological process of nitrification in soil was first shown by Schlosing and Muntz<sup>10</sup> and further confirmed by Warington<sup>11</sup> Frankland<sup>12</sup> and other workers. A large amount of work has been carried on by Dhar and collaborators<sup>13</sup> on the process of nitrification and they have established that the nitrification of ammonium salts or other nitrogenous compounds on various purely chemical solid surfaces and soil is attended with nitrate formation and nitrogen loss, and that it can take place under complete sterile conditions where no bacterial activities are possible. The oxidation is always more pronounced in the light than in the dark. The above experimental results are in complete agreement with the observations of Dhar and co-workers.

From the experimental results it is clear that the adding of phosphatic fertilizers to soil in presence of ammonium sulphate is beneficial from the point of view of the stability of the nitrogenous fertilizers. This seems to be due to the conversion of ammonium nitrite formed in the nitrification to calcium nitrite which is more stable than ammonium nitrite. The calcium ion is produced in the system containing calcium phosphate.

The results obtained by the authors are in agreement with some of the results obtained at Rothamsted<sup>14</sup> on the crop yield per acre on a soil treated with ammonium sulphate containing enough phosphate and potassium salt.

*Effect of Phosphates and potassium salts on the utilization of Nitrates by wheat plants*

BROAD BALK

Treatment	Crop yield per acre per annum		Nitrogen recovered in crops lb per annum	Nitrogen present as nitrate in drainage water during autumn p.p.	Percentage of nitrogen in soil	Nitrogen lost from soils lb per annum
	Grain	Straw				
Ammonium salts containing 86 lbs nitrogen abundant sup- plies of P and K salts	26.7	30.75	33.5	8.5	0.116	51.0
No and K salts	16.0	14.75	45.0	17.8	0.106	67.5

The above results clearly show that phosphates and potassium salts when applied along with ammonium sulphate to the soil markedly checks the nitrogen loss. Our results also prove that different varieties of phosphates can check the loss of nitrogen on adding ammonium sulphate to soils.

#### SUMMARY

1. When ammonium sulphate is added to the soil there is always a loss of nitrogen in the gaseous state attended with the formation of nitrites and nitrates.
2. When ammonium sulphate is added with different phosphates, the loss of nitrogen is appreciably checked.
3. The loss is checked to a greater extent in the case of dicalcium phosphate, tricalcium phosphate, rock phosphate and bone meal.
4. The loss of nitrogen is always more prominent in light than in the dark, and the oxidation is always pronounced in light than in the dark.
5. The checking of the loss of nitrogen seems to be due to the conversion of ammonium nitrite formed in the nitrification to the calcium nitrite which is more stable than ammonium nitrite. The calcium ion is produced in the system containing calcium phosphate.
6. Rothamsted field experiments have shown that the application of phosphates and potassium salts markedly checks the nitrogen loss.

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# INFLUENCE OF DECOMPOSING ORGANIC MATTER ON THE AVAILABILITY OF PHOSPHATES

By N. R. DHAR and B. K. DHAR

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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Hester<sup>1</sup> has shown that the addition of suitable organic matter to the sandy truck soils of the coastal plains of U. S. A. may so reduce the fixation of phosphate to an unavailable form that optimum yields of crops may be produced with much less superphosphate than is commonly used. Jensen<sup>2</sup> found that the addition of organic matter to a soil increases the solubility of both lime and phosphoric acid 30—100 %. Collings<sup>3</sup> has reported that soils that contain high percentage of organic matter are better suited for the application of ground rock phosphate than are soils containing medium or low percentage of organic matter.

Dhar<sup>4</sup> has recently stated that when phosphates are mixed with organic substances like green manure, farmyard manure, straw, leaves and other plant residues they can act as partial substitutes for nitrogenous fertilizers by fixing atmospheric nitrogen and supply available phosphate and potash and trace elements and build up the soil fertility by increasing the humus status.

In view of the above observations we have studied the influence of decomposing organic matter on the availability of phosphates, when different phosphates like monocalcium, dicalcium, tricalcium phosphate, phosphate rocks, phosphates of iron, aluminium and magnesium and superphosphates are added to different soils. The decomposable organic matter used in our experiments are cane sugar, molasses and wheat straw.

## EXPERIMENTAL PROCEDURE

0.25 %  $P_2O_5$  in the form of abovementioned forms of phosphates were added to two varieties of Indian soils. The moisture content of all the experiment was maintained at 20 %. After definite intervals of time the soil samples were taken for the analysis of available phosphate and exchangeable calcium. The available phosphate was determined by the method of Dyer<sup>5</sup> and the further procedure was carried out according to the method of Pemberton<sup>6</sup> (method adopted by A.O.A.C.). The exchangeable calcium was determined by neutral ammonium acetate leaching method (*vide* Official and Tentative methods of analysis of the Association of Official Agricultural Chemistry, 6th Ed, 1945, p 14). The further procedure was followed as mentioned by Piper.<sup>7</sup>

Available Phosphate may be referred to as 1 % citric acid soluble phosphate.

*Experimental results with molasses*

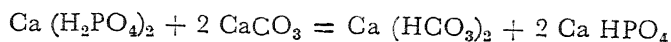
Treatment	Available $P_2O_5$ in grams per 100 grams of soil			Exchangeable calcium in milli-equivalents		
	0 days	50 days	100 days	0 days	50 days	100 days
Soil + molasses (1 %)	0.0329	0.0331	0.0334	22.9	24.2	24.8
„ + „ + phosphate rock of Trichinopoly I }	0.0356	0.0398	0.0448	23.4	28.6	30.7
„ + „ + phosphate rock of Behar }	0.0382	0.0442	0.0471	23.0	27.9	31.4
„ + „ + phosphate rock of Trichi. II }	0.0084	0.0420	0.0469	23.1	27.3	30.5
„ + „ + phosphate rock of kudada }	0.0395	0.0496	0.0598	24.0	29.4	31.5
„ + „ + mono- calcium phosphate. Ca ( $H_2PO_4$ ) $_2$ . $H_2O$ }	0.2234	0.1896	0.1843	36.4	30.5	25.8
„ + „ + dicalcium phosphate. $CaHPO_4$ . $2H_2O$ }	0.2104	0.2112	0.2134	34.5	34.4	34.4
„ + „ + tricalcium phosphate $Ca_3(PO_4)_2$ }	0.1820	0.2089	0.2100	28.8	31.7	36.8
„ + „ + superphosphate	0.2432	0.1592	0.1564	35.7	28.4	24.6
„ + „ + bone meal	0.1200	0.1269	0.2098	23.6	25.4	27.5

*Experimental results with Straw*

Treatment	Available $P_2O_5$ in grams per 100 grams of soil			Exchangeable calcium in milli-equivalents		
	0 days	90 days	180 days	0 days	90 days	180 days
Soil + 1 % wheat straw	0.1708	0.1709	0.1711	39.5	39.7	39.6
„ + „ + phosphare rock of Behar }	0.1906	0.1925	0.1936	40.2	43.1	44.5
„ + „ + phosphate rock of Trichinopoly }	0.1896	0.1989	0.2001	40.0	43.8	44.7
„ + „ + mono- calcium phosphate }	0.4860	0.2284	0.2120	58.2	50.8	46.2
„ + „ + dicalcium phosphate }	0.3245	0.3356	0.3358	49.4	50.6	53.2
„ + „ + tricalcium phosphate }	0.2916	0.3204	0.3412	48.8	52.6	54.7
„ + „ + superphos- phate	0.3960	0.2742	0.2242	55.4	45.2	43.0

It has been clearly brought out in the foregoing experimental observations that the availability of phosphate appreciably increases in presence of the organic matter, specially cane sugar molasses which is undergoing decomposition and oxidation in the soil. The carbonic acid and small amounts of organic acids produced from molasses are helpful in the conversion of tricalcium phosphate into dicalcium phosphate and small amounts of monocalcium phosphates which are more soluble in water than the tricalcium phosphate. Hence the decomposition of organic matter with the lapses of time leads to the formation of larger amounts of dicalcium phosphate and small amounts of monocalcium phosphates at the expense of tricalcium phosphate and the available phosphate of the system increases as recorded above.

On the other hand when monocalcium or superphosphate is added to the soil the available phosphate which high in the beginning decreases in course of time. This is due to the fact the monocalcium phosphate  $\text{Ca}_2(\text{H}_2\text{PO}_4)_2$  reacts with calcium carbonate  $\text{CaCO}_3$  according to the following equation in the soil :



Thus the soluble monocalcium phosphate when added to the mixture is converted into the dicalcium phosphate which is much less soluble than the monocalcium phosphate as is recorded in the following table :—

Substance	Formula	Solubility in 100 part of water at 0°C
Monocalcium phosphate	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	4.0 at 15°C
Dicalcium phosphate	$\text{CaHPO}_4 \cdot 2 \text{H}_2\text{O}$	0.028
Tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	0.0013

It is interesting to note that with cane sugar molasses the availability of phosphates appreciably increases when rock phosphate or tricalcium phosphate is added to the system. This is certainly due to the conversion of the tricalcium phosphate present in the rock into the dicalcium and monocalcium state. When the dicalcium phosphate is used in these experiments instead of tricalcium phosphate there is hardly any increase in the availability with lapse of time. In a recent publication Dhar<sup>8</sup> has emphasised that in soils under neutral conditions the carbonic acid can readily convert tricalcium into more soluble dicalcium phosphate. But as the first dissociation constant of phosphoric acid is much higher than the dissociation of carbonic acid the formation of monocalcium phosphate by interaction of carbonic acid on dicalcium and tricalcium phosphate is difficult and hence the amount of monocalcium salt is likely to be small.

Dissociation constants of carbonic and phosphoric acids are :—

Carbonic acid :  $\text{H}_2\text{CO}_3$  : dissociation constant =  $3 \times 10^{-7}$

$$\frac{\text{H}^+ \times \text{HCO}_3'}{\text{H}_2\text{CO}_3} = K_1 = 3 \times 10^{-7} \text{ (first dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{CO}_3''}{\text{HCO}_3'} = K_2 = 6 \times 10^{-11} \text{ (second dissociation constant)}$$

Phosphoric acid :  $\text{H}_3\text{PO}_4$  : dissociation constant =  $9 \times 10^{-8}$

$$\frac{\text{H}^+ \times \text{H}_2\text{PO}_4'}{\text{H}_3\text{PO}_4} = K_1 = 1.1 \times 10^{-2} \text{ (first dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{HPO}_4''}{\text{H}_2\text{PO}_4'} = K_2 = 2 \times 10^{-7} \text{ (second dissociation constant)}$$

$$\frac{\text{H}^+ \times \text{PO}_4'''}{\text{HPO}_4''} = K_3 = 3.6 \times 10^{-13} \text{ (third dissociation constant)}$$

Consequently when dicalcium phosphate is used in these experiments the available phosphate has not appreciably increased in course of time because the formation of the more soluble monocalcium phosphate is difficult from the dicalcium phosphate by the action of carbonic acid and other weak organic acids produced in the system.

In all these cases containing tricalcium phosphate and phosphate rock there is an appreciable increase in exchangeable calcium due to the formation of dicalcium phosphate and small amounts of monocalcium salt from tricalcium phosphate. On the other hand with superphosphate or monocalcium phosphate there is no increase of exchangeable calcium or even in some cases there is a decrease of the exchangeable calcium due to the conversion of soluble monocalcium phosphate into less soluble dicalcium phosphate in course of time.

It is interesting to record here that the total phosphate per acre present in this soil is 1769 lbs per acre, out of which about  $\frac{1}{2}$  i.e., 736.9 lbs of  $\text{P}_2\text{O}_5$  per acre is in the available condition. When 1% molasses is added to this soil, the available phosphorus increases to 748.16 lbs per acre i.e., there is an increase of 11.2 lbs of  $\text{P}_2\text{O}_5$  per acre in 100 days. If to the same system different doses of phosphates are added the amount of available phosphate is increased even in the case of sparingly soluble phosphate rocks collected from different parts of India. It is clear, therefore, that in Allahabad where the average temperature of the soil in the year is  $26^\circ\text{C}$  and the soils have a tendency to be on the alkaline side the available phosphate even when powdered rock phosphate is added, increases markedly and it reaches the values 866.8 lb per acre to 1050.5 lbs per acre in 100 days, when molasses is added to such a system.

With straw which undergoes slower oxidation in the soil than molasses the production of carbonic acid in unit time is smaller than molasses. Hence, the increases of available phosphate on the addition of tricalcium phosphate or phosphate rocks is much less pronounced than in the case of molasses as a source of organic matter. In presence of monocalcium phosphate or superphosphate, due to the conversion of monocalcium phosphate to dicalcium phosphate which is less soluble, the available phosphate decreases in course of time as in the case of cane sugar molasses used as an organic matter.

Many investigators Baur,<sup>9</sup> Copland and Markla,<sup>10</sup> Gereetson,<sup>11</sup> Jensen,<sup>12</sup> have observed that organic matter increased the availability of soil phosphate and of rock phosphate which was added to the soil as a fertilizer.

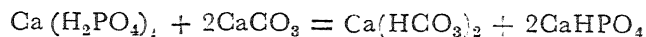
#### SUMMARY

1. Availability of phosphate appreciably increases in presence of the organic matter.



2. The decomposition of organic matter with the laps of time leads to the formation of larger amounts of dicalcium phosphate at the expense of tricalcium phosphate in the form of phosphate rock or as such.

3. When monocalcium phosphate or superphosphate are added to the soil, the available phosphate which is high in the beginning decreases in course of time, probably according to the following reaction :—



4. When dicalcium phosphate is added to the soil there is no appreciable change in the available phosphate, which is due to the fact that the first dissociation constant of phosphoric acid being much higher than the dissociation constant of carboric acid, the formation of monocalcium by the interaction of carboric acid on dicalcium and tricalcium phosphate is not possible.

5. In all the cases containing tricalcium phosphate or rock phosphate there is an appreciable increase in exchangeable calcium. When monocalcium or superphosphate is added there is decrease of exchangeable calcium.

6. When molasses is applied to the soil available phosphate increases from 735.9 lbs to 478.16 lbs per acre *i.e.*, there is an increase of 11.2 lbs of  $\text{P}_2\text{O}_5$  per acre in 100 days.

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# ADSORPTION OF $H_3PO_4$ , $H_2PO_4'$ , $HPO_4''$ AND $PO_4'''$ BY NORMAL SOIL

By N. R. DHAR and B. K. DHAR

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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In recent years a large amount of experimental work is being conducted in different in countries on the fixation phosphates and the availability of phosphate present the soil, or added to it in the form of commercial phosphate fertilizer. As fixation or reversion of soluble phosphates in the soil plays a very important role, we have carried on experiments on the fixation of soluble phosphate by a sample of Indian soil.

*Method.*—A quantity of 2.5 grms of the air dry soil was added to 90 m.l. of a solution containing HCl or NaOH (for the adjustment of desired pH values) and the mixture was left for 24 hours. On the next day 10 m.l. of the phosphate solution either in the form of  $H_3PO_4$ , or  $NaH_2PO_4$  or  $Na_2HPO_4$  or  $Na_3PO_4$  containing 376 m gm. of  $P_2O_5$  per 100 grms of the air dried soil were added and the contents were shaken for 4 hours in a mechanical shaker. After settling it was filtered and the  $P_2O_5$  was determined photo-electric colorimetrically by Warren and Pugh's method. Ultra filtration was carried out wherever necessary.

## EXPERIMENTAL RESULTS

Fixation of  $H_3PO_4$ ,  $H_2PO_4'$ ,  $HPO_4''$  and  $PO_4'''$  by the soil

pH	Percentage of $P_2O_5$ fixed as			
	$H_3PO_4$	$H_2PO_4'$	$HPO_4''$	$PO_4'''$
3	5.9	18.4	36.6	68.7
4	6.9	20.6	49.8	50.3
5	8.9	30.5	57.7	48.4
6	12.7	40.5	92.8	46.5
7	16.7	20.4	83.0	42.8
8	13.7	12.7	40.2	38.6
9	10.3	5.3	36.8	36.4
10	9.7	—	12.5	30.8

From the survey of the foregoing experimental results the fixation of phosphate ions depends a good deal on the pH of the medium. The greater the pH, the greater is the adsorption of phosphate ions. This seems to be due to the reaction of phosphate ions with calcium present in the soil leading to the formation of the tricalcium phosphate and dicalcium phosphate in the insoluble condition. It is further confirmed by the fact that the soil used for the experiments given

above shows a greater adsorption in the case  $\text{Na}_2\text{HPO}_4$  and  $\text{Na}_3\text{PO}_4$ . when they are in the medium at pH 6—7. In other words in neutral or alkaline media the soluble phosphate as superphosphate is converted chiefly into the dicalcium phosphate.

Steele<sup>2</sup> stated that the fixation of phosphates depends upon three conditions.

1. At pH 2—5 the reaction is due to the gradual formation of iron and aluminium ions and there precipitates as phosphate.

2. At pH 4.5—7.5 the phosphate seem to be fixed on the surfaces of calv minerals.

3. At pH 6—10 the phosphate is fixed by divalent cations.

Chatterji and Datta<sup>3</sup>, Ford,<sup>4</sup> Metzger,<sup>5</sup> Chandler,<sup>6</sup> Coleman,<sup>7</sup> Mattson,<sup>8</sup> Steele,<sup>9</sup> Bradfield,<sup>10</sup> Murphy,<sup>11</sup> Hillgardie, Scarseth,<sup>12</sup> Ravikovitch,<sup>13</sup> Midgley<sup>14</sup> and Kelley have advanced two possibilities in the case of phosphate fixation.

1. Fixation of phosphate mainly due to its precipitation by iron, alminium or calcium.

2. And adsorption of anion exchange also plays a prominent role in the phosphate fixation.

As the soil used is rich in total and exchangeable calcium, the fixation of phosphates takes place chiefly as sparingly soluble phosphates of calcium *i.e.*,  $\text{CaHPO}_4$  and  $\text{Ca}(\text{PO}_4)_2$ . As the fixation is maximum between pH 6—7 in all the phosphate solutions it clearly favours the formation of insoluble calcium phosphate Dean<sup>15</sup> fractionated the phosphorus contained in the slightly calcarous soil at Rothamstead and concluded that the phosphorus which had been applied as superphosphate had been retained as tricalcium phosphate or apatite.

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# EFFICIENCY OF ATMOSPHERIC NITROGEN FIXATION IN SOIL BY THE APPLICATION OF PHOSPHATE AND CALCIUM CARBONATE

By N. R. DHAR AND S. K. GHOSH

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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The use of phosphates in reinforcing manures is too well-known and Bear<sup>1</sup> has remarked that "Within limits phosphate fertilizers together with potash salts and lime can be substituted for nitrogen fertilizers. Their use stimulates the nitrogen fixing bacteria, both symbiotic and non-symbiotic to greater activity." Collings<sup>2</sup> has stated as follows: "Low crop yields are more often due to a lack of phosphoric acid than to a lack of any other nutrient. Phosphoric acid has often been called the "master key" to agriculture. Phosphoric acid appears to be concerned in the production of nucleoproteids and it appears that phosphoric acid influences the production of seed or grain more particularly than does nitrogen or potash." Sir John Russell<sup>3</sup> observes "... the phosphate applied to the soils may determine the course of history even in near future, specially in cold and wet countries."

From a survey of soils of different countries it appears that the nitrogen status of a soil is intimately connected with its phosphorus status. When the  $P_2O_5$  content is 1000 lbs per acre, the nitrogen content cannot be greater than 0.1%, but when the  $P_2O_5$  content becomes 2000 lbs per acre or more, the nitrogen content can go up to 0.2% or more. In other words, as the  $P_2O_5$  content of a soil increases, there is a marked increase of its nitrogen content. As a matter of fact, the increase in the nitrogen content appears to be more steep with increasing amounts of  $P_2O_5$  content in the soil. The fen soils of England and muck soils in New Jersey, which are rich in nitrogen (2.7--3% N) contain over 0.2%  $P_2O_5$ . The results of field trials conducted at Illinois Experiment Station<sup>4</sup> as well as Tennessee Experiment Station show that crops can give a good response to phosphatic fertilizers. Thorne (1907) [Soil Science by W. W. Weir, p. 400] and others have found that when manure is reinforced with 25 to 40 lbs of superphosphate per ton, its fertilizing efficiency is greatly enhanced, specially on soils that are deficient in available phosphorus. Dhar<sup>5</sup> has obtained marked nitrogen fixation in soil, both under sterile and unsterile condition, by the application of different doses of phosphates and calcium carbonate along with organic matter. Dhar<sup>6</sup> has also remarked that by the use of lime or calcium carbonate to an acid soil, not only the acidity decreases but also the carbonaceous matter undergoes more rapid

oxidation leading to greater fixation of atmospheric nitrogen in soils so that the C/N ratio decreases and its fertility increases. As the nitrogen fixation occurs even under completely sterile conditions by the application of phosphates and calcium carbonate, which is always more in light than in the dark, it can be concluded that the process of nitrogen fixation in soils is partly due to surface and photo-oxidation of all kinds of energy materials which is aided by phosphates and calcium carbonate.

In view of the above facts we have in this paper carefully studied the nitrogen fixation, both under sterile and unsterile conditions, effected by the slow oxidation of starch on soil surface by the application of disodium hydrogen phosphate and calcium carbonate in artificial light and in the dark. We have also compared the efficiency of this type of nitrogen fixation with the well-known industrial processes of nitrogen fixation.

#### EXPERIMENTAL

The sample of soil used was well crushed, air dried, screened through a 100 mesh sieve and carefully analysed for its mineral constituents. The chemicals used were A. R. reagents. For unsterile sets the reaction mixtures containing soil and starch along with and without known amounts of disodium hydrogen phosphate or calcium carbonate were placed in shallow enamelled dishes after adding distilled water to bring the moisture content to about 20%. The mixtures were stirred on alternate days to facilitate aeration. For sterile experiments the reaction mixtures were placed in long Jena glass test-tubes containing 10 mls of distilled water. The mixtures were sterilised in an autoclave for 4 hours at 20 lbs pressure. After sterilisation, a small portion of the material was taken out from each test-tube and plated for total bacterial count using Thornton's media. The mixtures thus autoclaved were found to be free from bacteria. Loss of nitrogen from soils during sterilisation as has been observed by Berthelot,<sup>7</sup> Pfeiffer<sup>8</sup> and Warmbold<sup>9</sup> was also taken into account. The concentration of disodium hydrogen phosphate and calcium carbonate in all cases was 0.25 gm  $P_2O_5$  as  $Na_2HPO_4$  and 0.25 gm  $CaCO_3$  respectively per 100 gms of the soil. The dishes and the test-tubes containing the reaction mixtures were exposed to light from a 60 watt electric bulb and kept at a distance of 3 ft from the bulb whilst for the experiments in dark similar sets kept at the same place were covered with thick black cloth. The mean temperature during the exposure was 25°C. After definite interval of time samples were taken out and analysed for their total carbon and total nitrogen. The total carbon and total nitrogen, in all cases, were estimated according to the method of Robinson, Mclean and Williams<sup>10</sup>. The following results were obtained :—

TABLE I

*Analysis of oven-dried soil*

In grams per cent.

pH of the soil=8.00

SiO <sub>2</sub>	Sesquioxide	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Total-C	Total-N	NH <sub>3</sub> -N	NO <sub>3</sub> -N
75.00	9.80	4.37	4.07	1.52	1.00	0.42	1.86	0.26785	0.00608	0.02030

TABLE 2

<i>Sterile</i>	<i>Light</i>		Average temperature-25°C		
	Per 100 gms of the mixture				
Period of exposure in days	Total carbon present in gm	Total carbon oxidized in gm	Total nitrogen present in gm	Grain in total nitrogen gm	Efficiency
	1 gm soil + 0.0225 gm Starch				
0	2.6227	0.0	0.2334	0.0	...
100	2.0950	0.5277	0.2608	0.0274	51.92
	1 gm soil + 0.0225 gm Starch + Phosphate				
0	2.6227	0.0	0.2334	0.0	...
100	1.9000	0.7227	0.2885	0.0551	76.24
	1 gm soil + 0.0225 gm Starch + Calcium carbonate				
0	2.6227	0.0	0.2334	0.0	...
100	1.8582	0.7645	0.2931	0.0597	78.09

TABLE 3

<i>Sterile</i>	<i>Dark</i>		Average temperature-25°C		
	1 gm soil+0.0225 gm Starch				
0	2.6227	0.0	0.2334	0.0	...
100	2.2906	0.3321	0.2429	0.0095	28.60
	1 gm soil+0.0225 gm Starch + Phosphate				
0	2.6227	0.0	0.2334	0.0	...
100	2.0882	0.5345	0.2600	0.0266	49.77
	1 gm soil+0.0225 gm Starch+ Calcium carbonate				
0	2.6227	0.0	0.2334	0.0	...
100	2.0461	0.5766	0.2632	0.0298	51.68

TABLE 4

<i>Unsterile</i>	<i>Light</i>			Average temperature = 25°C	
	Per 100 gms of the mixture				
	100 gms soil + 2.25 gms Starch.				
0	2.5650	0.0	0.2620	0.0	—
60	1.9980	0.5670	0.2951	0.0331	58.4
100	1.8620	0.7030	0.3289	0.0669	95.2
	100 gms soil + 2.25 gms Starch + Phosphate				
0	2.5525	0.0	0.2606	0.0	—
60	1.7520	0.8005	0.3333	0.0727	90.2
100	1.6623	0.8902	0.3571	0.0965	108.4
	100 gms soil + 2.25 gms Starch + Calcium carbonate				
0	2.5587	0.0	0.2613	0.0	—
60	1.7100	0.8487	0.3408	0.0765	93.7
100	1.6200	0.9387	0.3636	0.1023	108.9

TABLE 5

*Unsterile**Dark*

Average temperature = 25°C

Per 100 gms of the mixture

Period of exposure in days	Total carbon present in gm	Total carbon oxidized in gm	Total nitrogen present in gm	Gain in total nitrogen in gm	Efficiency
100 gms soil + 2.25 gms Starch					
0	2.5650	0.0	0.2620	0.0	—
60	2.1300	0.4350	0.2788	0.0168	38.6
100	2.0110	0.5540	0.2995	0.0375	67.7
100 gms soil + 2.25 gms Starch + Phosphate					
0	2.5525	0.0	0.2606	0.0	—
60	1.9500	0.6025	0.3030	0.424	70.4
100	1.8619	0.6906	0.3226	0.620	89.8
100 gms soil + 2.25 gms Starch + Calcium carbonate					
0	2.5587	0.0	0.2613	0.0	—
60	1.8600	0.6987	0.3113	0.0500	71.6
100	1.8180	0.7407	0.3334	0.0721	97.3

A perusal of the foregoing tables shows that there is appreciable oxidation of carbon and fixation of nitrogen, under both sterile and unsterile conditions, when starch is allowed to undergo slow oxidation in air on soil surface both in light as well as in the dark. There is, in general, greater oxidation of organic matter and more nitrogen fixation in light than in the dark.

It will be also seen from these results that in all cases the presence of either disodium hydrogen phosphate or calcium carbonate increases the oxidation of organic matter and efficiency of nitrogen fixation per gram of carbon oxidized. The oxidation, however, is greater in presence of calcium carbonate than with disodium hydrogen phosphate. The following tables (6 and 7) have been compiled from tables (2 to 5) for the percentage increase in the efficiency of nitrogen fixation both by the application of disodium hydrogen phosphate and calcium carbonate in light as well as in the dark after 100 days of exposure.

TABLE 6

*Sterile*

Condition	Percentage increase in the efficiency of nitrogen fixation	
	With phosphate	With calcium carbonate
Light	46.84	50.38
Dark	73.98	80.69

TABLE 7

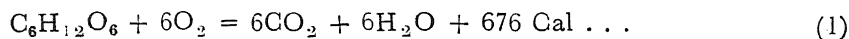
*Unsterile*

Condition	Percentage increase in the efficiency of nitrogen fixation	
	With phosphate	With calcium carbonate
Light	13.86	14.39
Dark	32.64	43.72

The foregoing results clearly show that in the presence of either disodium hydrogen phosphate or calcium carbonate there is an increase in the efficiency of of nitrogen fixation.

As we have observed marked nitrogen fixation and the effect of both disodium hydrogen phosphate and calcium carbonate in increasing the efficiency of nitrogen fixation under completely sterile condition we are forced to conclude that nitrogen fixation has taken place without any bacterial agency. It is interesting to record that as soon as some protien or amino acid or ammonium salts are produced in the process of fixation of atmospheric nitrogen, these nitrogenous compounds also undergo oxidation causing the formation and decomposition of the unstable substance ammonium nitrite. Hence, nitrogenous compounds formed cannot remain for a long time and is thus lost. Thus, when the of loss of nitrogen during nitrification is prominent, the apparent fixation becomes less efficient. Hence, the fixation of nitrogen is opposed by the phenomenon of loss of nitrogen involved in nitrification caused by the formation and decomposition of the unstable substance ammonium nitrite. The decomposition of ammonium nitrite is well-known to be catalysed by hydrogen ions, but due to the small dissociation constants of either carbonic acid or phosphoric acid, the presence of their salts in the system considerably check the increment in the hydrogen ion concentration in the system by the oxidation of organic matter in the soil. Hence, it can be inferred that by the use of calcium carbonate or phosphate not only the acidity of the system decreases but also the carbonaceous matter undergoes more rapid oxidation causing greater fixation of nitrogen. It is well-known that alkali favours oxidation of carbohydrates and retards the decomposition of ammonium nitrite. Again in presence of large amounts of phosphate in the system more or less stable phosphoproteins are formed by the combination of proteins and phosphorus compounds. These compounds seem to resist nitrification and ammonification and loss of nitrogen better than proteins alone. These appear to be important reasons why we have obtained greater oxidation of organic matter and higher efficiency of nitrogen fixation both in presence of disodium hydrogen phosphate or calcium carbonate both in light as well as in the dark.

*Mechanism of nitrogen fixation.* The main chemical changes involved in photosynthesis in plants, is the decomposition of water into H and OH by absorption of energy obtained from the slow oxidation of carbohydrates, cellulose, lignin, fats, coals, etc. The atomic hydrogen thus formed can reduce molecular nitrogen to ammonia. The ammonia in its turn undergoes nitrification and forms nitrogen by the joint action of atmospheric oxygen and OH radical. When light acts on the system, whether from the sun or artificial source, a part of the light energy is utilised and causes greater fixation of nitrogen. The same mechanism may be applicable in symbiotic nitrogen fixation. The energy relationships in the reaction involved during nitrogen fixation are given in the following equations :—



Hence, for fixing 14 grams of nitrogen and forming ammonia by the interaction of molecular nitrogen and atomic hydrogen by the decomposition of water



336 Cals are needed. Hence, from the oxidation of a gram molecule of glucose according to equation (1)  $\frac{14 \times 676}{336}$  grams of nitrogen can be fixed under ideal conditions. In other words, 390 mgms of nitrogen should be fixed per gram of carbon oxidized. In our experiments under unsterile condition phosphates or calcium carbonate using starch as energy material in 100 days, about 108 mgms. of nitrogen are fixed per gram of carbon oxidized in light (see table 4). Hence, the efficiency of this type of nitrogen fixation in soil is  $\frac{108 \times 100}{390}$  i.e., 27.7% in light using starch along with phosphates or calcium carbonate. In sterile condition the efficiency in 100 days is  $\frac{77 \times 100}{390}$  i.e. 19.75% in light (see table 2). In dark the efficiency both under unsterile and sterile condition is less. This, therefore, shows that there is a marked light effect in this type of nitrogen fixation which is certainly more efficient than the industrial processes of nitrogen fixation. In the Haber-Bosch process which is generally adopted at present in most countries the yield of ammonia is usually 8% of the initial nitrogen concentration. Physicists and industrialists have concluded that the average efficiency of the process is 10%. The efficiency of the arc process of nitrogen fixation is not more than 5%. In cyanamide process of nitrogen fixation, which requires only  $\frac{1}{3}$  of the energy which is required for the arc process, the cyanamide has to be converted into ammonia for industrial purposes and hence this method has not been generally utilised except in Germany.

Therefore, the use of either disodium hydrogen phosphate or calcium carbonate in increasing the efficiency of this type of nitrogen fixation by utilising chemical energy will lead to marked nitrogen fixation not only to the acid soils of the world but also to the soils rich in organic matter as found in many places of Europe and America. The use of phosphates and calcium carbonate is of double benefit, *firstly*, as agents for increasing the nitrogen status of a soil and, *secondly*, by their vital importance as plant nutrients.

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# FIXATION OF ATMOSPHERIC NITROGEN IN SAND BY THE SLOW OXIDATION OF SOME ALIPHATIC ACIDS

By N. R. DHAR and S. K. GHOSH

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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In a number of publications Dhar<sup>1,2</sup> and co-workers have observed that when organic materials like glucose, cane sugar, starch, glycerol, etc., which contain no nitrogen, are allowed to undergo slow oxidation in air, both in sterile and unsterile conditions in presence of water and the surfaces of various chemically pure insoluble substances or soil, there is a fixation of atmospheric nitrogen, which is more in light than in the dark. They<sup>3</sup> have further shown that plant residues, farmyard manure and even peat, lignite and bituminous coal, etc. which contain nitrogen not only add the nitrogen they contain but are also able to fix nitrogen. Mukerji and Dhar<sup>4</sup> have observed marked fixation of nitrogen under sterile conditions, more in light than in the dark. Dhar has, therefore, concluded from these results that fixation of nitrogen is possible by the oxidation of organic matter even in sterile condition proving thereby that the nitrogen fixation occurring in soils is also possible by purely chemical agencies. Miller<sup>5</sup> has pointed out that higher nitrogen fixation, as occurs in light than in dark, cannot be explained from microbiological theory. Evidences of photo-chemical theory put forward by Dhar have since been confirmed by other workers at different places. H. M. Kerr at Brisbane (Australia) working with some sandy loam types of soils found that fixation of nitrogen in light is double than that in the dark. Recently, Jensen in Lyngby (Denmark), Stockli in Zurich and Lemoigne in Paris, have emphasised that *Azotobacter* does not play a big role in the nitrogen status of soils.

The present investigation deals with the fixation of atmospheric nitrogen by the slow oxidation of some aliphatic acids in contact with sterile and unsterile sand samples in artificial light and in the dark. The acids were specially chosen to keep the medium acidic so that the possibility of bacterial action in nitrogen fixation may be far remote.

## EXPERIMENTAL

The sample of sand used was crushed, air dried, screened through a 30 mesh sieve and completely analysed for its mineral constituents. The organic acids used were A. R. reagents free from nitrogen. For unsterile sets, the reaction mixture (containing sand and different doses of the acids) were placed in dishes after adding distilled water to bring the moisture content to 20%. The mixtures were stirred on alternate days to facilitate aeration. After definite intervals samples were taken out and analysed for their total carbon and total

nitrogen contents. Electrical conductivity and hydrogen ion concentration of the aqueous extracts of the mixture of sand and the acids were also measured.

For sterile experiments the reaction mixtures were placed in Jena glass test-tubes containing 10 mls of distilled water. These mixtures were sterilised in an autoclave for 4 hours at 20 lbs pressure. After sterilisation, a small portion of the material was taken out from each test-tube plated for total bacterial count using Thornton's media. The mixtures thus autoclaved were found to be perfectly free from bacteria. The dishes or tubes containing the reaction mixture were exposed to light from a 60 watt bulb and were kept at a distance of 3 feet from the electric bulb. For experiments in the dark, similar sets were kept at the same place covered with thick black cloth. The temperature was recorded on every day. After a known interval of time, the test-tubes containing the reaction mixtures were dried on a water bath and aliquot portions of them were analysed for total carbon and total nitrogen. The total carbon, in all cases was estimated by the method adopted by Walkley and Black<sup>6</sup> and total nitrogen by the Kjeldahl method.<sup>7</sup>

The sterilisation as well as the incubation of sterile soils is likely to lead to a loss of nitrogen as has been shown first by Berthelot<sup>8</sup>, later by Pfeiffer<sup>9</sup> and Warmbold.<sup>10</sup> To avoid this type of error, a test-tube containing 10 gms of sand alone with 10 mls distilled water was also kept along with these test-tubes in the autoclave to ascertain the loss of nitrogen if any. It was also found that during autoclaving there was a slight decrease in the total nitrogen content as recorded below :--

Nitrogen content of sand before sterilisation	Nitrogen content after sterilisation	Loss of Nitrogen on sterilisation
0.00893 %	0.00833 %	6.71 %

Hence, the final reading obtained after sterilisation was utilised as the zero reading for all such sterilised set of experiments. The results of both, sterile and unsterile sets, are as follows :--

TABLE No. 1

Percentage composition of the oven-dried sand

Total—C	= 0.02730
Total—N	= 0.00893
CaO	= 2.408
K <sub>2</sub> O	= 0.990
P <sub>2</sub> O <sub>5</sub>	= 0.044
pH of the sand	= 8.3

TABLE No. 2

In grams per cent.

Period of exposure in days	Total carbon in the acid	Carbon in the acid oxidised	Total nitrogen present	Gain in total nitrogen	Efficiency
10 gms Sand + 0.3125 gm Tartaric acid—Sterile					Light
0	1.0000	0.0	0.00807	0.0	...
220	0.88448	0.11552	0.00940	0.00133	11.51
10 gms Sand + 0.3125 gm Tartaric acid					Dark
0	1.00000	0.0	0.00807	0.0	...
220	0.09176	0.09824	0.00860	0.00053	5.31

TABLE No. 3

In grams per cent.

Period of exposure in days	Total carbon in the acid	Carbon in the acid oxidised	Total nitrogen present	Gain in total nitrogen	Efficiency
	10 gms Sand	0.2916 gm Citric acid			Light
0	1.00000	0.0	0.00809	0.0	...
220	0.93073	0.06927	0.00896	0.00087	12.56
	10 gms Sand + 0.2916 gm Citric acid				Dark
0	1.00000	0.0	0.00809	0.0	...
220	0.97740	0.02260	0.00826	0.00017	7.52

TABLE No. 4

	10 gms Sand + 0.525 gm Oxalic acid				Light
0	1.00000	0.0	0.00791	0.0	...
220	0.92720	0.07280	0.00897	0.00196	26.9
	10 gms Sand + 0.525 gm Oxalic acid				Dark
0	1.00000	0.0	0.00791	0.0	...
220	0.96880	0.03120	0.00830	0.00039	12.00

TABLE No. 5.

In grams per cent

4 gms of the sample taken and made up to 100 mls

Period of exposure in days	Total carbon in the acid	Carbon in the acid oxidized	Total nitrogen present	Gain in total nitrogen	Efficiency	pH	Electric conductivity in mls
	200 gms sand + 6.25 gms Tartaric acid—Unsterile						Light
0	1.00000	0.0	0.00865	0.0	...	...	$3.47 \times 10^{-4}$
187	0.52810	0.47190	0.01515	0.00650	13.79	5.20	$5.81 \times 10^{-4}$
305	0.31264	0.68736	0.02083	0.01218	17.72	5.50	$0.70 \times 10^{-4}$
	200 gms Sand + 6.25 gm Tartaric acid						Dark
0	1.00000	0.0	0.00865	0.0	...	...	$3.47 \times 10^{-4}$
187	0.55280	0.44720	0.01136	0.00271	6.06	5.10	$9.67 \times 10^{-4}$
305	0.34576	0.65424	0.01470	0.00605	9.25	5.30	$1.12 \times 10^{-4}$

TABLE No. 6

	200 gms Sand + 5.8332 gms Citric acid						Light
0	1.00000	0.0	0.00868	0.0	...	...	$2.71 \times 10^{-4}$
187	0.52820	0.47180	0.0351	0.00483	10.24	5.20	$6.96 \times 10^{-4}$
305	0.24426	0.75574	0.01923	0.01055	13.96	5.60	$0.63 \times 10^{-4}$
	200 gms Sand + 5.8332 gms Citric acid						Dark
0	1.00000	0.0	0.00868	0.0	...	...	$2.71 \times 10^{-4}$
187	0.63940	0.36060	0.01111	0.00243	6.74	5.00	$8.15 \times 10^{-4}$
305	0.38109	0.61891	0.01450	0.00582	9.40	5.30	$1.41 \times 10^{-4}$

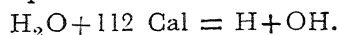
TABLE No. 7

Period of exposure in days	Total carbon in the acid	Carbon in the acid oxidized	Total nitrogen present	Gain in total nitrogen	Efficiency	pH	Electric conductivity in mls
200 gms Sand + 10.5 gms Oxalic acid							
Light							
0	1.00000	0.0	0.00848	0.0	...	...	...
187	0.009143	0.9087	0.01191	0.00343	3.78	5.20	$7.84 \times 10^{-4}$
305	0.006166	0.93834	0.01670	0.00822	8.76	5.50	$0.63 \times 10^{-4}$
200 gms Sand + 10.5 gms Oxalic acid							
Dark							
0	1.00000	0.0	0.00848	0.0	...	...	...
187	0.19048	0.80952	0.00963	0.00115	1.42	5.10	$9.13 \times 10^{-4}$
305	0.11679	0.88321	0.01316	0.00468	5.29	5.40	$0.87 \times 10^{-4}$

Results from the foregoing tables show that when aliphatic acids like tartaric, citric and oxalic are left in air in contact with sand and moisture, there is always a decrease in the carbon content resulting in a concomitant increase in the nitrogen content of the system both under sterile and unsterile conditions. This effect in all cases is more marked in light than in the dark. Again, in the case of unsterile sets, the efficiency of nitrogen fixation, *i.e.* the nitrogen fixed in milligrams per gram of carbon oxidized, increases with lapse of time. We have also obtained similar types of results by using different concentrations of these acids with the same amount of sand. However, the efficiency of nitrogen fixation obtained by us with these acids is rather lower than that obtained by Dhar and collaborators with other energy materials. It seems that with acids as source of energy, the medium being highly acidic (pH-5), a part of the nitrogen fixed may be lost. It is interesting to find that in the case of oxalic acid (in unsterile condition) the oxidation of this acid being too fast, the efficiency of nitrogen fixation is low. Tables 5 to 7 also show that electrical conductivity of the aqueous extracts containing different acids first increases and then rapidly falls off with time. It seems that these organic acids first from soluble salts by the interaction of the inorganic materials present in the said, and finally the conductivity falls off because of the oxidation both of the acids and its salts. That there is greater oxidation is further shown by the decrease in the acidity of the aqueous extracts with lapse of time. These results, therefore, confirm that these acids undergo slow oxidation on sand surface. It will also be of interest, to notice from these results that efficiency of nitrogen fixation, both under unsterile and sterile conditions, is practically of the same order, although the rate of oxidation of the acids is smaller in sterile condition than in unsterile sets. This, therefore shows that the existence or nonexistence of bacteria does not materially affect the nitrogen status of the sand. Hence, the mechanism of nitrogen fixation seems to be identical in sterile and in unsterile condition.

#### *Mechanism of Nitrogen Fixation*

Dhar (1933) postulated that the important photo-chemical reaction during photosynthesis in plants is the decomposition of water by absorption of light according to the following equations :—



The hydrogen atom thus formed reduces the carbonic acid adsorbed on the leaves forming formaldehyde. In recent years this view of the mechanism

of photosynthesis has been supported by the use of carbonic acid containing isotopic carbon. In explaining nitrogen fixation the best mechanism seems to be the same, i.e. the decomposition of water into H and OH absorption of energy obtained from the slow oxidation of organic acids or other energy rich materials in contact with soil or sand by atmospheric oxygen at the ordinary temperature, as the carbon of the system steadily decreases with time. The hydrogen atoms thus formed have always the possibility of coming in contact with the nitrogen molecules (perhaps adsorbed on the sand surface) forming ammonia. The ammonia in its turn undergoes nitrification and forms nitrates by the joint action of atmospheric oxygen and OH radical. When light acts on the system, whether from the sun or artificial source, a part of the light energy is absorbed and causes greater fixation of nitrogen. The same mechanism may also be applicable in symbiotic nitrogen fixation. Hence, nitrogen fixation seems to be a catalysed surface reaction induced by the oxidation of energy rich materials, and water plays a prominent part in the mechanism of nitrogen fixation as postulated above. It is well-known that in absence of moisture no nitrogen fixation takes place in soil or sand.

We have thus been able to put forward an alternate theory of chemical and photo-chemical nature to explain the phenomenon of nitrogen fixation in soil or sand.

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# LOSS OF NITROGEN IN BACTERIAL NITRIFICATION AND DENITRIFICATION

By N. R. DHAR and MAYA SHANKER LAL

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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Nitrogen being an elusive substance, when fixed or added as manure does not remain for long in the soil under ordinary conditions. A part of the added nitrogen may be utilized by the plant and the rest may be lost as gaseous nitrogen or may be lost by leaching. It has been suggested by a number of investigators that the gaseous loss of nitrogen from soil is a factor of great importance to agriculture and causes a real danger to soil fertility.

The researches of Lipman and Blair,<sup>1</sup> Russell and Richards,<sup>2</sup> Shutt and others show that nitrogen in the gaseous state is lost from the soil when the conditions are favourable for oxidation. Experiments at Rothamsted fields reveal that when 200 lbs of nitrogen per acre are added, approximately 65% are lost without benefit to the soil or the crop.

In previous publications<sup>3</sup> Dhar has shown that marked losses of nitrogen take place during the nitrification of organic and inorganic manures in soil culture. Recently Dhar and Pant<sup>3</sup> have observed a great loss of nitrogen during the bacterial nitrification of ammonium salts and organic manures.

In this paper it is proposed to undertake a systematic study of gaseous loss of nitrogen during bacterial nitrification and denitrification of different substances both organic and inorganic in liquid cultures.

## EXPERIMENTAL

The following medium was prepared for the growth of bacteria :—

Potassium phosphate ( $K_2HPO_4$ )	...	1.0 gm
Sodium chloride	...	2.0 gm
Ferrous sulphate	...	0.4 gm
Magnesium carbonate	excess (0.5 gm per 100 c.c.)	
Distilled water		1 litre

Fifty c.c. of this solution was taken in 300 c. c. conical flasks. The flasks were then plugged with absorbent cotton wool and were sterilized in an autoclave for 30 mts at 15 lbs of pressure per sq. inch.

A 10% solution of  $(NH_4)_2SO_4$  was prepared and sterilized separately. After sterilization the nitrogen content was estimated. From one to 10 c.c. of this solution was added (according to the nitrogen content) to the various flasks containing the sterilized media, by a sterile pipette. One gram of fresh garden soil was inoculated into each flask and flasks were kept for inoculation at  $35^\circ (\pm 0.5)$  for a period of seven weeks. 10% solutions of  $NH_4Cl$ ,  $NH_4NO_3$ ,  $NaNO_3$ ,  $CO(NH_2)_2$  and creatine were prepared separately, sterilized, nitrogen content estimated and used in the similar manner as in the case of  $(NH_4)_2SO_4$ . In order to compare the percentage loss of nitrogen in  $(NH_4)_2SO_4$ ,  $NH_4Cl$ ,  $NH_4NO_3$ ,  $NaNO_3$ ,  $CO(NH_2)_2$

and creatine, the nitrogen states was kept equal in all the experiments. The flasks with their contents were shaken twice a week. The experiments were set up in duplicates.

#### METHOD OF ANALYSIS

The solution after incubation was leached with 5% solution of KCl to a known volume.

(i) *Ammoniacal nitrogen*.—For estimating ammoniacal nitrogen in the leachate, an aliquot was treated with 5 gms of pure KCl and 5 gms of pure MgO and 50 c.c. water and distilled for one hour on direct Bunsen burner flame. The ammonia set free was absorbed in known amount of 0.02N HCl in conical flasks. The content of the flask was boiled for 5 mts (in order to remove CO<sub>2</sub>), cooled and titrated against 0.02N NaOH using 2 to 3 drops of mixed indicator (0.125 gm Methyl. red + 0.0825 gm Methylene blue dissolved in 100 c.c. alcohol (ethyl.)).

1 c.c. of  $\frac{N}{10}$  HCl used up  $\equiv$  0.0014 gm of nitrogen.

(ii) *Nitric nitrogen*.—The residue from which the ammonia was removed by the previous procedure, was treated with one gram of Devarda's alloy (free from ammonia and nitrate) and 25 c. c. of 2% sodium hydroxide solution and left overnight for the reduction of nitrite and nitrate to ammonia. When the reduction was complete, the ammonia set free was determined as in the first stage by titration. To ensure accuracy the nitrogenous impurities in the various reagents were estimated and due deductions were made.

(iii) *Total nitrogen*.—An aliquot of the leachate was taken in Kjeldahl flask. 2 to 3 drops of conc. H<sub>2</sub>SCl<sub>4</sub> was added to acidify the solution. The content in the Kjeldahl were evaporated to dryness on electric water bath and cooled. 30 ml. of conc. H<sub>2</sub>SO<sub>4</sub> containing 1 gm of salicylic acid was added to it and mixed. The flask was allowed to stand for 30 minutes and was shaken frequently.

5 grams of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 10 H<sub>2</sub>O (crystals) were added to the flask, which was then kept on the digestion rack and was heated for 5 mts over low flame until all danger from frothing had passed. It was cooled and 5 gms of K<sub>2</sub>SO<sub>4</sub> or anhydrous sodium sulphate, 0.1 to 0.3 gm of CuSO<sub>4</sub> was added and heated over a low flame until all dangers from frothing has passed. Increased the flame and continued with the digestion, distillation and titration as in the regular Kjeldahl process. A blank determination was also conducted to correct for the amount of nitrogen in the reagent.

#### RESULTS

##### *Analysis of soil used for inoculation*

pH (soil + 2.5 water) 8.4

pH (soil + 2.5 water) 8.4				
1. Loss on ignition			...	3.68%
2. Silica (HCl insoluble)			...	74.54%
3. Fe <sub>2</sub> O <sub>3</sub>			...	4.48%
4. Sesquioxide			...	12.47%
5. Silica/sesquioxide ratio			...	5.9
6. Percentage of :				
	CaO	MgO	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
	2.93	1.83	0.2872	0.192
7. Available PO <sub>4</sub> (1% citric acid)			...	0.0273%
8. Total carbon			...	0.239%
9. Total N			...	0.0436%
10. C/N			...	5.4
11. NH <sub>3</sub> --N			...	0.00024%



TABLE I

 $(\text{NH}_4)_2\text{SO}_4$  as source of Nitrogen

Period of incubation = 7 weeks

Average temperature  $35 (\pm 0.5^\circ\text{C})$ 

No.	Original N-content (gm)	$\text{NH}_3\text{-N}$ observed (gm)	$\text{NO}_3\text{-N}$ observed (gm)	Total-N left (gm)	Total-N lost (gm)	% loss of N
1	0.2448	0.1197	0.0360	0.1557	0.0891	36.41
2	0.1836	0.0613	0.0314	0.0927	0.0909	49.55
3	0.1224	0.0285	0.0243	0.0528	0.0697	56.91
4	0.0612	0.0082	0.0129	0.0211	0.0402	60.67
5	0.0306	0.0035	0.0059	0.0094	0.0212	59.32
6	0.0300	0.0019	0.0046	0.0065	0.0235	78.29
7	0.0245	0.0006	0.0027	0.0033	0.0212	86.55

TABLE II

 $\text{NaNO}_3$  as source of Nitrogen

Period of incubation = 7 weeks

Average temperature  $35^\circ (\pm 0.5^\circ)$ 

No.	Original N-content (gm)	$\text{NH}_3\text{-N}$ observed (gm)	$\text{NO}_3\text{-N}$ observed (gm)	Total N left (gm)	Total-N lost (gm)	% loss of N
1	0.2448	0.0038	0.2233	0.2271	0.0176	7.41
2	0.1836	0.0011	0.1663	0.1674	0.0162	8.83
3	0.1224	0.0023	0.1083	0.1106	0.0118	9.66
4	0.0612	0.0026	0.0507	0.0533	0.0079	12.98
5	0.0306	0.0019	0.0246	0.0265	0.0041	13.65
6	0.0300	0.0029	0.0228	0.0257	0.0044	14.61
7	0.0245	0.0043	0.0113	0.0206	0.0039	16.26

TABLE III

 $\text{NH}_4\text{Cl}$  as source of Nitrogen

Period of incubation = 7 weeks

Average temperature  $35^\circ (\pm 0.5^\circ)$ 

No.	Original N-content (gm)	$\text{NH}_3\text{-N}$ observed (gm)	$\text{NO}_3\text{-N}$ observed (gm)	Total-N left (gm)	Total-N lost (gm)	% loss of N
1	0.2448	0.1311	0.0340	0.1651	0.0796	32.56
2	0.1836	0.0711	0.0281	0.0992	0.0844	45.92
3	0.1224	0.0380	0.0216	0.0596	0.0628	51.37
4	0.0612	0.0130	0.0098	0.0228	0.0384	62.84
5	0.0306	0.0060	0.0042	0.0108	0.0198	64.72
6	0.0300	0.0041	0.0031	0.0072	0.0228	76.14
7	0.0245	0.0027	0.0019	0.0046	0.0199	81.49

TABLE IV

CO $\leq$ NH as source of Nitrogen

Period of incubation=7 weeks Average temperature 35°(±0.5°)						
No.	Original N- content (gm)	NH <sub>3</sub> -N observed (gm)	NO <sub>3</sub> -N observed (gm)	Total-N left (gm)	Total-N lost (gm)	% loss of N
1	0.2448	0.0319	0.0618	0.1804	0.0645	26.34
2	0.1836	0.0302	0.0454	0.1245	0.0591	32.20
3	0.1224	0.0104	0.0197	0.0743	0.0482	39.37
4	0.0612	0.0092	0.0063	0.0350	0.0262	42.85
5	0.0306	0.0063	0.0054	0.0156	0.0150	49.05
6	0.0300	0.0035	0.0049	0.0130	0.0170	56.97
7	0.0245	0.0024	0.0031	0.0089	0.0156	63.74

TABLE V

NH<sub>4</sub>NO<sub>3</sub> as source of Nitrogen

Period of incubation=7 weeks Average temperature 35°(±0.5°)						
No.	Original N- content (gm)	NH <sub>3</sub> -N observed (gm)	NO <sub>3</sub> -N observed (gm)	Total-N left(gm)	Total-N lost (gm)	% loss of N
1	0.2448	0.0588	0.1191	0.1779	0.0669	27.36
2	0.1836	0.0474	0.0629	0.1103	0.0733	39.92
3	0.1224	0.0337	0.0342	0.0679	0.0545	44.57
4	0.0612	0.0074	0.0236	0.0310	0.0302	49.32
5	0.0306	0.0047	0.0087	0.0134	0.0172	56.25
6	0.0300	0.0038	0.0056	0.0094	0.0206	68.88
7	0.0245	0.0023	0.0035	0.0058	0.0187	76.53

TABLE VI

NH<sub>2</sub> C(=NH)N(CH<sub>3</sub>) CH<sub>2</sub>COOH as source of Nitrogen

Period of incubation 7 weeks Average temperature 35° (±0.5°).						
No.	Original N content (gm)	NH <sub>3</sub> -N observed (gm)	NO <sub>3</sub> -N observed (gm)	Total-N left (gm)	Total-N lost(gm)	% loss of N
1	0.2448	0.0093	0.0247	0.1932	0.0516	21.10
2	0.1836	0.0065	0.0195	0.1397	0.0439	23.92
3	0.1224	0.0048	0.0123	0.0802	0.0422	34.55
4	0.0612	0.0041	0.0095	0.0396	0.0216	35.39
5	0.0306	0.0021	0.0038	0.0182	0.0148	40.79
6	0.0300	0.0023	0.0024	0.0152	0.0148	49.35
7	0.0245	0.0008	0.0019	0.0102	0.0143	58.51

## DISCUSSION

The foregoing experimental results show that on bacterial nitrification of  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{CO}(\text{NH}_2)_2$  and creatine and denitrification of  $\text{NaNO}_3$ , under conditions quite favourable for the growth of bacteria, there is marked loss of nitrogen even in seven weeks time. The nitrogen introduced at the start of the experiment and the nitrogen recovered after it are not balanced. On the other hand people, upto now, have been believing that the conversion of ammonia to nitrate through bacterial agency is quantitative as is clear from the following statement of Russell :—

“... Nitrate production is confined to one or two organisms only at each stage, and the end result is a single product quantitatively equivalent to the original ammonia.”

The results obtained by us clearly point out that even during the bacterial nitrification of ammonium or other nitrogenous compounds which can be ammonified, there is always a loss of nitrogen. It has been also found that greater the initial nitrogen content, greater is the loss of nitrogen though the percentage loss of nitrogen increases with dilution. Another interesting fact brought out from these results is that the loss of nitrogen and the nitrification is much more in the case of ammonium salts than with other nitrogenous substances. This is in accordance with the following statement of Vishwanath :—

“There is always a greater loss of N and greater nitrification with ammonium salts than with the farmyard or green manures.”

Urea shows an easy nitrification though the loss recorded in this case is less than that of ammonium salts. Losses in case of ammonium nitrate seems to be comparatively less than  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{NH}_4\text{Cl}$ . A closer examination of the result shows that the amount of nitrate formed is always less than that introduced originally as if the ammonium part of the substance undergoes oxidation as in the case of  $(\text{NH}_4)_2\text{SO}_4$  but nitrate counterpart of the substance undergoes slight reduction. Loss of nitrogen is found to be minimum with  $\text{NaNO}_3$  because the nitrification is not possible and hence the formation and decomposition of the unstable substance ammonium nitrite is also small. In substance like creatine, the loss of N is much less during the same period and the amount of nitrate formed is also less as is clear from the following table :—

TABLE VII

Loss of N with different substances during the bacterial nitrification with the same amount of original nitrogen

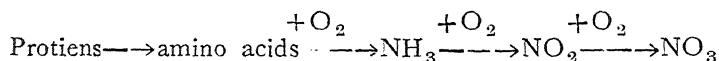
No.	Substance	Nitrate formed in mgm	Loss of N (%)
1	$(\text{NH}_4)_2\text{SO}_4$	24.3	56.91
2	$\text{NH}_4\text{Cl}$	21.6	51.37
3	Urea	19.7	39.37
4	Creatine	12.3	34.55

In substances other than ammonium salts, the nitrogen is present in complex form and hence is very slowly nitrified. It seems that in the presence of carbonaceous matter along with nitrogenous compounds the velocity of oxidation of compounds and the amount of nitrogen loss is also decreased. The presence of organic matter also seems to hinder the activity of the nitrifying organisms.

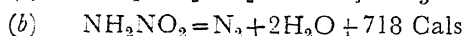
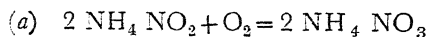
## MECHANISM OF NITROGEN LOSS

The mechanism of reactions involved in liquid cultures appears to be the same as in the soil cultures when nitrogenous manures are added to the soil under the field or laboratory conditions.

The loss of nitrogen during the nitrification has been explained by Dhar and co-workers on the viewpoint that when nitrogenous fertilizers or protein are added to the soil the following changes take place :—



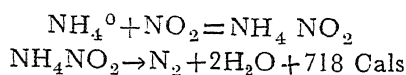
In this processes the unstable substance ammonium nitrite is formed as an intermediate product and can undergo oxidation and decomposition as follows :—



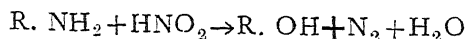
The second chemical change is more prominent than the first as it is highly exothermal and hence there is considerable loss of nitrogen.

The loss of nitrogen during denitrification has been variously explained. Nitrates, during denitrification are reduced to nitrites, oxides of nitrogen, nitrogen gas and ammonia, depending on the type of bacteria and the condition in which they are working.

$2\text{NaNO}_3 \rightleftharpoons 2\text{NaNO}_2 + \text{O}_2$  This nitrite formed can react with  $\text{NH}_4^+$  and form nitrogen gas as follows :—



There is also a possibility that nitrogen gas may be liberated by a chemical process under acid condition for nitrites can react with amines to give nitrogen gas, thus



There is no evidence however that this reaction is of any importance in the soil.

## SUMMARY

1. Loss of nitrogen has been observed during the process of bacterial nitrification of ammonium salts and other nitrogen substances like urea and creatine and denitrification of sodium nitrate.

2. The loss of nitrogen is greater from the oxidation of ammonium salts than with other nitrogenous substances. The ammonium salts are more easily nitrified than the other substances used.

3. The greater the initial nitrogen content, greater is the loss of, though the percentage loss nitrogen increases with dilution.

4. The loss of nitrogen in all these cases has been explained on the view point that during nitrification and denitrification, ammonium nitrite is formed which decomposes into nitrogen gas which accounts for the observed loss of nitrogen from the soil recorded so glaringly by all the workers throughout the world.

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## EFFECT OF PHOSPHATES IN NITROGEN FIXATION USING BENTONITE MINERAL AS THE SURFACE

By N. R. DHAR and M. M. RAI

*Sheila Dhar Institute of Soil Science, University of Allahabad*

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Dhar and co-workers<sup>1</sup> have carried on a large amount of research work on the problem of nitrogen fixation for over twenty five years. They have used different kinds of energy rich materials like glucose, sucrose, starch, plant residues, leaves, farmyard manure, lignite, coals, etc. in the soil or sand. They have observed that when these materials undergo oxidation on the surface of the soil or sand, there is a concomitant fixation of atmospheric nitrogen. It is interesting to note that this type of fixation is always more pronounced in light than in the dark. They have also shown that nitrogen fixation is possible with chemically pure inorganic surfaces like  $\text{ZnO}$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Ni}_2\text{O}_3$ ,  $\text{CoO}$ ,  $\text{MnO}_2$ ,  $\text{BaSO}_4$ , ferric phosphate and calcium phosphates, etc., using various energy materials in light as well as in dark, both in sterile and unsterile conditions.

Bentonites are clays that are produced by the alteration of volcanic ash in Situ. Such clays are chiefly composed of montmorillonite clay minerals. They are highly plastic, colloidal and swelling clays. It has the unique property of swelling to many times its original volume when placed in water. Since bentonite is an important group of clay minerals found in soil, we have carried on experiments on this type of nitrogen fixation using Kashmir bentonite as the surface and glucose, sucrose, starch as the energy rich materials.

### EXPERIMENTAL

Kashmir bentonite was powdered in a mechanical grinder and sieved through a 100 mesh sieve. For the unsterile experiments an amount of oven-dried bentonite was taken, to which the energy materials and for phosphates were added; the total weight of the reaction mixture became 100 grams. Two corresponding sets were thus set up in enamel plates of 24 cm diameter and one was exposed to artificial light of 100 watt electric bulb placed at a vertical distance of two feet and the other set was kept in the dark covered with a thick black cloth. The moisture content of the system was maintained at 20–25% by adding distilled water. The content of the plates were thoroughly stirred with glass rod to facilitate aeration. Samples were taken out after 40 and 60 days after powdering the content of the dish well with the help of a mortar and pestle.

For the sterile experiments an amount of oven-dried bentonite was taken, to which when energy materials and/or phosphates were added, the total weight of the mixture became 2.0 grams. This mixture was taken in test-tubes and 10 c.c. of distilled water was added and the test-tubes were plugged with cotton wool and autoclaved at 15 lbs pressure per square inch for 30 minutes. After autoclaving one set was exposed to light and the other kept in dark, as in the unsterile

sets. The test-tubes were occasionally shaken to facilitate aeration. The test-tubes were taken after exposure of 150 and 180 days and dried on waterbath after the addition of few drops of dilute sulphuric acid, in order to avoid loss of ammonia during drying. After drying the test-tubes were very carefully broken in a mortar and pestle and transferred to Kjeldahl flasks for the carbon and nitrogen determinations. The sterile tubes have been tested twice for any bacterial contamination and have been found to be free from it.

The total carbon of the system was determined by the method described by Robinson, Mclean and Williams.<sup>2</sup> The total nitrogen was determined by the modified Kjeldahl method<sup>3</sup> to include the nitrate nitrogen. In order to measure small changes in the value of nitrogen  $\frac{N}{50}$  acid and alkali were used. The indicator used for the titration was mixed methyl red.<sup>4</sup> The experimental results have been recorded in the following tables :—

TABLE No. 1

Analysis of the Kashmir bentonite (oven-dry basis)

Moisture	16.44%
Silica	54.44%
Loss on ignition	9.32%
Sesquioxide	22.64%
Fe <sub>2</sub> O <sub>3</sub>	2.242%
Al <sub>2</sub> O <sub>3</sub>	20.40%
CaO	2.44%
MgO	4.77%
K <sub>2</sub> O	0.44%
P <sub>2</sub> O <sub>5</sub>	0.042%
pH (1:2.5)	7.5
Exchangeable calcium (NaCl method)	46.0 m.e./100 gm of material
Exchangeable magnesium (NaCl method)	20.0 m.e./100 gm of material
Exchangeable potassium	1.46 m.e./100 gm of material

TABLE No. 2

Bentonite + 0.5% Carbon as Sucrose + water

Days of exposure	Total carbon unoxidized %	Total carbon oxidized %	Total nitrogen in milligrams	Gain in total nitrogen in mgms	Efficiency
Exposed to artificial light—Unsterile					
0	0.5796	...	4.35	...	...
40	0.2899	0.2897	24.68	20.33	70.20
60	0.1870	0.3926	30.80	26.45	67.36
Dark—Unsterile					
0	0.5796	...	4.35	...	...
40	0.2239	0.3557	16.02	11.67	32.81
60	0.1381	0.4415	18.58	14.23	32.24
Light—Sterile					
0	0.5796	...	4.35	...	...
150	0.2038	0.3758	17.95	13.60	36.21
180	0.1472	0.4324	19.84	15.49	35.82
Dark—Sterile					
0	0.5786	...	4.35	...	...
150	0.4688	0.1108	5.18	0.83	7.54
180	0.4684	0.1112	5.15	0.80	7.23

TABLE No. 3

Bentonite + 0.5% Carbon as Sucrose + 0.5% P<sub>2</sub>O<sub>5</sub> as Monocalcium phosphate + water

Days of exposure	Total carbon unoxidized %	Total carbon oxidized %	Total nitrogen in mgms	Gain in total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5789	...	4.35	...	...
40	0.2293	0.3496	31.21	26.86	76.81
60	0.1755	0.4034	34.76	30.41	75.38
Dark—Unsterile					
0	0.5789	...	4.35	...	...
40	0.1806	0.3983	19.93	15.58	39.12
60	0.1114	0.4675	22.03	17.68	87.81
Light—Sterile					
0	0.5789	...	4.35	...	...
150	0.1884	0.3905	19.98	15.63	40.02
180	0.1592	0.4397	21.82	17.47	39.72
Dark—Sterile					
0	0.5789	...	4.35	...	...
150	0.4627	0.1162	5.49	1.14	9.87
180	0.4619	0.1170	5.46	1.11	9.57



TABLE No. 4

Bentonite + 0.5% Carbon as Sucrose + 0.5%  $P_2O_5$  as Dicalcium phosphate + water

Days of exposure	Total carbon unoxidized%	Total carbon oxidized%	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5786	...	4.35	...	...
40	0.2130	0.3656	39.00	34.65	94.81
60	0.1164	0.4622	47.07	42.72	92.42
Dark—Unsterile					
0	0.5786	...	4.35	...	...
40	0.1630	0.4156	23.55	19.20	46.20
60	0.0960	0.4826	26.46	22.11	45.82
Light—Sterile					
0	0.5786	...	4.35	...	...
150	0.1732	0.4054	24.02	19.67	48.52
180	0.1346	0.4440	25.43	21.08	47.48
Dark—Sterile					
0	0.5786	...	4.35	...	...
150	0.4582	0.1204	5.90	1.55	12.94
180	0.4576	0.1210	5.88	1.53	12.72

TABLE No. 5

Bentonite + 0.5% Carbon as sucrose + 0.5%  $P_2O_5$  as Tricalcium phosphate + water

Days of exposure	Total carbon unoxidized%	Total carbon oxidized%	Total nitrogen in mgms	Gain of total nitrogen in mgm	Efficiency
Light—Unsterile					
0	0.5787	...	5.35	...	...
40	0.1812	0.3975	37.07	32.72	82.32
60	0.0892	0.4895	43.77	39.42	80.53
Dark—Unsterile					
0	0.5787	...	4.35	...	...
40	0.1400	0.4386	22.04	17.69	40.34
60	0.0512	0.5275	26.12	21.77	41.27
Light—Sterile					
0	0.5787	...	4.35	...	...
150	0.1441	0.4346	22.35	18.00	41.42
180	0.0832	0.4955	24.44	20.09	40.56
Dark—Sterile					
0	0.5787	...	4.35	...	...
150	0.4485	0.1302	5.75	1.40	10.84
180	0.4475	0.1312	5.64	1.29	9.90

TABLE No. 6

Bentonite + 0.5% Carbon as Sucrose + 0.5%  $P_2O_5$  as Ferric phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5785	...	4.35	...	...
40	0.2496	0.3289	28.13	23.78	72.30
60	0.1778	0.4007	32.72	28.37	70.82
Dark—Unsterile					
0	0.5785	...	4.35	...	...
40	0.1929	0.3856	18.56	14.21	36.85
60	0.1153	0.4627	20.98	16.63	35.93
Light—Sterile					
0	0.5785	...	4.35	...	...
150	0.2016	0.3769	18.39	14.04	37.25
180	0.1454	0.4331	20.30	15.95	36.84
Dark—Sterile					
0	0.5785	...	4.35	...	...
150	0.4655	0.1130	5.33	0.98	8.72
180	0.4650	0.1135	5.31	0.96	8.52

TABLE No. 7

Bentonite + 0.5% Carbon as Sucrose + 0.5%  $P_2O_5$  as Aluminium phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5787	...	4.35	...	...
40	0.2692	0.3095	26.48	22.13	71.50
60	0.1830	0.3957	31.80	27.45	69.36
Dark—Unsterile					
0	0.5787	...	4.35	...	...
40	0.2092	0.3695	17.10	12.75	34.50
60	0.1330	0.4457	19.57	15.22	34.12
Light—Sterile					
0	0.5787	...	4.35	...	...
150	0.2028	0.3759	18.05	13.70	36.44
180	0.1460	0.4327	19.93	15.58	36.00
Dark—Sterile					
0	0.5787	...	4.35	...	...
150	0.4675	0.1112	5.24	0.89	8.02
180	0.4671	0.1116	5.22	0.87	7.82

TABLE No. 8

Bentonite+0.5% Carbon as Glucose+water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5794	...	4.19	...	...
40	0.2838	0.2956	23.86	19.67	66.53
60	0.1760	0.4034	30.80	26.61	65.98
Dark—Unsterile					
0	0.5794	...	4.19	...	...
40	0.2806	0.2988	13.40	9.21	30.82
60	0.1709	0.4085	15.68	11.49	28.12
Light—Sterile					
0	0.5794	...	4.19	...	...
150	0.1834	0.3960	17.82	13.63	34.42
180	0.1268	0.4526	19.50	15.31	33.84
Dark—Sterile					
0	0.5794	...	4.19	...	...
150	0.4581	0.1213	5.02	0.83	6.92
180	0.4577	0.1217	5.01	0.82	6.75

TABLE No 9

Bentonite+0.5% Carbon as Glucose+0.5% P<sub>2</sub>O<sub>5</sub> as Mono-calcium phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5787	...	4.19	...	...
40	0.2731	0.3056	27.38	23.19	75.89
60	0.1670	0.4117	35.28	31.09	75.52
Dark—Unsterile					
0	0.5787	...	4.19	...	...
40	0.2690	0.3097	16.62	12.43	40.12
60	0.1622	0.4165	20.29	16.10	38.84
Light—Sterile					
0	0.5787	...	4.19	...	...
150	0.1692	0.4095	20.37	16.18	39.52
180	0.1188	0.4599	22.04	17.85	38.82
Dark—Sterile					
0	0.5787	...	4.19	...	...
150	0.4567	0.1220	5.19	1.00	8.21
180	0.4562	0.1225	5.16	0.97	8.01

TABLE No. 10

Bentonite + 0.5 % c as glucose + 0.5% P<sub>2</sub>O<sub>5</sub> as Di-calcium phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5784	...	4.19	...	...
40	0.2681	0.3103	32.19	28.00	90.24
60	0.1624	0.4160	41.16	36.97	88.90
Dark—Unsterile					
0	0.5784	...	4.19	...	...
40	0.2648	0.3136	19.01	14.82	47.28
60	0.1576	0.4208	23.08	18.89	44.90
Light—Sterile					
0	0.5784	...	4.19	...	...
150	0.1528	0.4256	24.43	20.24	47.55
180	0.1142	0.4642	25.95	21.76	46.87
Dark—Sterile					
0	0.5784	...	4.19	...	...
150	0.4544	0.1240	5.66	1.47	11.95
180	0.4538	0.1246	5.63	1.44	11.62

TABLE No. 11

Bentonite + 0.5% Carbon as Glucose + 0.5% P<sub>2</sub>O<sub>5</sub> as Tri-calcium phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5785	...	4.19	...	...
40	0.2110	0.3675	34.03	29.84	81.71
60	0.1408	0.4377	39.30	35.11	80.20
Dark—Unsterile					
0	0.5785	...	4.19	...	...
40	0.1869	0.3916	21.02	16.83	42.96
60	0.1288	0.4497	22.54	18.35	40.82
Light—Sterile					
0	0.5785	...	4.19	...	...
150	0.1239	0.4546	22.62	18.43	40.52
180	0.0628	0.5157	24.82	20.63	40.00
Dark—Sterile					
0	0.5785	...	4.19	...	...
150	9.4467	0.318	5.57	1.38	10.52
180	0.4460	0.1325	5.49	1.30	9.85

TABLE N. 12

Bentonite+0.5% Carbon as Glucose+0.5%  $P_2O_5$  as Ferric phosphate+water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5783	...	4.19	...	...
40	0.2756	0.3027	26.75	22.56	74.52
60	0.1696	0.4087	32.93	28.74	70.30
Dark—Unsterile					
0	0.5783	...	4.19	...	...
40	0.2716	0.3067	15.30	11.11	36.24
60	0.1636	0.4147	19.05	14.86	35.83
Light—Sterile					
0	0.5783	...	4.19	...	...
150	0.1814	0.3969	18.16	13.97	35.21
180	0.1254	0.4529	19.88	15.69	34.64
Dark—Sterile					
0	0.5783	...	4.19	...	...
150	0.4566	0.1217	5.07	0.88	7.31
180	0.4561	0.1222	5.04	0.85	7.01

TABLE No. 13

Bentonite+0.5% Carbon as Glucose+0.5%  $P_2O_5$  as Aluminium phosphate+water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5785	...	4.19	...	...
40	0.2788	0.2997	24.69	20.50	68.42
60	0.1728	0.4057	32.11	27.92	68.80
Dark—Unsterile					
0	0.5785	...	4.19	...	...
40	0.2769	0.3016	14.88	10.69	35.42
60	0.1680	0.4105	18.24	14.05	34.22
Light—Sterile					
0	0.5785	...	4.19	...	...
150	0.1823	0.3962	18.94	13.75	34.71
180	0.1258	0.4527	19.54	15.35	33.90
Dark—Sterile					
0	0.5785	...	4.19	...	...
150	0.4570	0.1215	5.04	0.85	7.05
180	0.4565	0.1220	5.03	8.84	6.92

TABLE No. 14

Bentonite+0.5% Carbon as starch+water					
Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5795	...	4.52	...	...
40	0.4378	0.1427	14.99	10.47	73.40
60	0.2610	0.3185	27.44	22.92	71.96
Dark—Unsterile					
0	0.5795	...	4.52	...	...
40	0.3445	0.2350	13.17	8.65	36.82
60	0.1440	0.4355	19.95	15.43	35.42
Light—Sterile					
0	0.5795	...	4.52	...	...
150	0.3594	0.2201	13.06	8.54	38.82
180	0.2982	0.2813	15.20	10.68	37.94
Dark—Sterile					
0	0.5795	...	4.52	...	...
150	0.5263	0.0532	4.98	0.46	8.72
180	0.5255	0.0540	4.97	0.45	8.52

TABLE No. 15

Bentonite+0.5% Carbon as Starch+0.5% P <sub>2</sub> O <sub>5</sub> as Mono-calcium phosphate+water					
Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5787	...	4.52	...	...
40	0.3892	0.1895	19.20	14.68	77.48
60	0.2222	0.3565	31.69	27.17	76.21
Dark—Unsterile					
0	0.5787	...	4.52	...	...
40	0.2780	0.3007	16.37	11.85	39.42
60	0.1200	0.4587	22.32	17.80	38.80
Light—Sterile					
0	0.5787	...	4.52	...	...
150	0.3234	0.2553	15.45	10.93	42.84
180	0.2638	0.3149	17.73	13.21	41.96
Dark—Sterile					
0	0.5787	...	4.52	...	...
150	0.5155	0.0632	5.14	0.62	9.92
180	0.5136	0.0651	5.15	0.63	9.74

TABLE No. 16

Bentonite + 0.5% Carbon as starch + 0.5%  $P_2O_5$  as Di-calcum phosphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5784	...	4.52	...	...
40	0.3409	0.2375	26.48	21.96	92.46
60	0.1914	0.3870	36.57	32.05	88.82
Dark—Unsterile					
0	0.5784	...	4.52	...	...
40	0.2293	0.3491	20.96	16.44	47.12
60	0.1089	0.4695	25.85	21.32	45.40
Light—Sterile					
0	0.5784	...	4.52	...	...
150	0.3156	0.2628	17.90	13.38	50.91
180	0.2472	0.3312	20.99	16.47	49.72
Dark—Sterile					
0	0.5784	..	4.52	...	...
150	0.5092	0.0692	5.45	0.93	13.52
180	0.5082	0.0702	5.44	0.92	13.15

TABLE No. 17

Bentonite + 0.5% Carbon as starch + 0.5%  $P_2O_5$  as Tri-calcium phpsphate + water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5785	...	4.52	...	...
40	0.3087	0.2698	26.87	22.35	82.80
60	0.1798	0.3987	36.59	32.07	80.42
Dark—Unsterile					
0	0.5785	...	4.52	...	...
40	0.2049	0.3736	20.04	15.52	41.56
60	0.0810	0.4975	24.76	20.24	40.70
Light—Sterile					
0	0.5785	...	4.52	...	...
150	0.2654	0.3131	18.41	13.89	44.35
180	0.1824	0.3961	21.95	17.43	44.00
Dark—Sterile					
0	0.5785	...	4.52	...	...
150	0.5031	0.0754	5.35	0.83	11.12
180	0.5017	0.0768	5.36	0.84	11.02

TABLE No. 18

Bentonite+0.5% Carbon as starch+0.5% P<sub>2</sub>O<sub>5</sub> as Ferric phosphate+water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5783	...	4.52	...	...
40	0.4096	0.1687	17.48	12.96	76.80
60	0.2392	0.3391	29.79	25.27	74.52
Dark—Unsterile					
0	0.5783	...	4.52	...	...
40	0.3084	0.2699	15.97	10.45	38.72
60	0.1296	0.4487	21.31	16.79	37.42
Light—Sterile					
0	0.5783	...	4.52	...	...
150	0.3500	0.2283	13.62	9.10	39.86
180	0.2965	0.2818	15.63	11.11	39.42
Light—Sterile					
0	0.5783	...	4.52	...	...
150	0.5243	0.0540	5.00	0.48	9.05
180	0.5231	0.0552	5.01	0.49	8.92

TABLE No. 19

Bentonite+0.5% Carbon as starch+0.5% P<sub>2</sub>O<sub>5</sub> as Aluminium phosphate+water

Days of exposure	Total Carbon unoxidized %	Total Carbon oxidized %	Total nitrogen in mgms	Gain of Total nitrogen in mgms	Efficiency
Light—Unsterile					
0	0.5785	...	4.52	...	...
40	0.4268	0.1517	15.87	11.35	74.83
60	0.2478	0.3307	28.63	24.11	72.92
Dark—Unsterile					
0	0.5785	...	4.52	...	...
40	0.3203	0.2582	14.25	9.73	37.72
60	0.1380	0.4405	20.75	16.23	36.84
Light—Sterile					
0	0.5785	...	4.52	...	...
150	0.3580	0.2205	13.17	8.65	39.24
180	0.2970	0.2815	15.43	10.91	38.76
Dark—Sterile					
0	0.5785	...	4.52	...	...
150	0.5249	0.0536	4.99	0.47	8.91
180	0.5241	0.0544	4.98	0.46	8.65



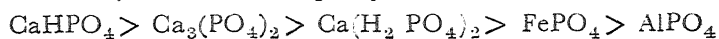
## DISCUSSION

From the foregoing tables it is clear that in all the unsterile sets, the rate of carbon oxidation is greater in dark than in light. This difference is possibly due to the fact that a considerable amount of the energy material is used by the micro-organisms for their multiplication. It is also well-known that light inhibits the growth of microbial population and darkness favours it. On the other hand the nitrogen fixation in the system exposed to light is much higher than the corresponding set kept in dark. This clearly indicates the possibility of photo-chemical nitrogen fixation in light.

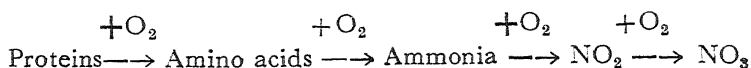
In the sterile sets we find that the rate of carbon oxidation is more in light than in the dark and the efficiency of nitrogen fixation, *i.e.*, mgms of nitrogen fixed/gram of carbon oxidized is always more in light than in the dark sets.

When the energy materials are added to the surface, they are slowly oxidized with liberation of energy and this energy is utilised in fixing the atmospheric nitrogen on the surface under suitable conditions. In natural conditions, the energy materials are oxidized on the surface as such by the photo-chemical action of light and also by the micro-organisms but under sterile conditions, only the photo-chemical action is responsible for the oxidation of the energy materials. Hence from our experiments under unsterile and sterile conditions, it is clear that the extent of photo-chemical nitrogen fixation is appreciably marked in all the cases.

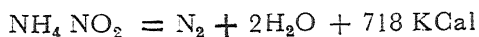
When different phosphate such as monocalcium phosphate, dicalcium phosphate, tricalcium phosphate, ferric-phosphate and aluminium phosphate were added in the system, along with energy materials, the rate of carbon oxidation invariably increases and along with this the amount of nitrogen fixed also increases and hence the efficiency is always higher than those without phosphates. The order of efficiency with different phosphates is as follows:—



The reasons for this pronounced increase in nitrogen fixation is not far to seek. As soon as some protein, amino acid and ammonium salts are formed in the system due to the fixation of nitrogen, these nitrogenous compounds undergo oxidation and nitrification aided by light as follows:—



In the course of these reactions there is always a possibility of the formation and decomposition of the unstable substance  $\text{NH}_4\text{NO}_2$  which readily breaks up into water and gaseous nitrogen which is lost



It is clear therefore that the process of nitrogen fixation is always opposed by the phenomenon of nitrogen loss as described above. Hence the apparent efficiency of nitrogen fixation, *i.e.*, the amount of nitrogen fixed per gram of carbon oxidized appears to fall off when ammonification and nitrification takes place. But in presence of phosphates there is always a possibility of the formation of more or less stable phospho-proteins and nucleo-proteins by the combination of proteins with phosphorus compounds. These compounds seem to resist ammonification and nitrification better than proteins alone. This appears to be one of

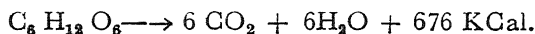
the reasons why the efficiency of nitrogen fixation appears larger in presence of phosphates.

Again it is well-known that the decomposition of  $\text{NH}_4\text{NO}_2$  is accelerated in presence of acids. When the energy materials undergo oxidation some organic acids may be produced in the system which accelerates the decomposition of  $\text{NH}_4\text{NO}_2$  leading to loss of nitrogen. But in presence of phosphates which act as buffer, due to the fact that its second and third dissociation constants are small, the increase in the  $\text{H}^+$  concentration of the system is not possible. Hence the efficiency of nitrogen fixation is more in presence of phosphates than in its absence.

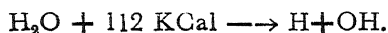
Moreover the incorporation of calcium phosphates introduces calcium ions in the system and this might be forming calcium nitrite by reacting with ammonium nitrite, which is an intermediate product in the nitrification and ammonification. Calcium nitrite being more stable than ammonium nitrite, a marked check in the loss of nitrogen would occur and the net result would appear to be an increase in the efficiency of nitrogen fixation.

#### MECHANISM OF NITROGEN FIXATION

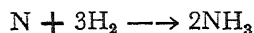
When the energy materials like glucose, sucrose, starch undergo oxidation on the surface of the bentonite mineral, they liberate large amount of energy according to the following equation :—



The energy thus liberated is much more than the energy required for the decomposition of water molecule into H and OH.



The atomic hydrogen thus formed combines with molecular nitrogen perhaps absorbed in the surface of bentonite and forms ammonia according to following equation :—



The ammonia thus formed undergoes further oxidation into  $\text{NO}_2$  and  $\text{NO}_3$ .

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